THE EVOLUTION OF CORAL SNAKE MIMICRY

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A dissertation submitted to the faculty at the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Biology.

Chapel Hill
2019

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ABSTRACT

Christopher Kemal Akcali: The evolution of coral snake mimicry
(Under the direction of David Pfennig)

Batesian mimicry—when individuals of a palatable species (the "mimic") gain the selective advantage of reduced predation because they resemble a toxic species (the "model") that predators avoid—is a classic example of the power of natural selection to drive adaptation. Although evolutionary biologists have long known about Batesian mimicry, many aspects of its evolution remain unclear.

My first chapter evaluated whether—and in which direction—Batesian mimicry has evolved in a natural population of mimics following extirpation of their model. Specifically, I examined whether the precision of coral snake mimicry has evolved among scarlet kingsnakes (*Lampropeltis elapsoides*) from a region where their model, the eastern coral snake (*Micrurus fulvius*), recently (1960) went locally extinct. I found that *L. elapsoides* has evolved more precise mimicry. These results suggest that selection imposed on mimics by predators has generated an "evolutionary momentum" towards precise mimicry after models became extirpated.

My second chapter examined the causes of geographic variation in the resemblance of Batesian mimics to their models. I characterized geographic variation in mimetic precision among four Batesian mimics of coral snakes to test hypotheses for geographic variation in mimicry. I found no evidence that geographic variation in mimicry in any species was a result of selective trade-offs between mimicry and thermoregulation or relaxed selection depending on mimic body size. I obtained evidence in support of the breakdown hypothesis—that mimicry is less precise when there is a paucity of models.

My third chapter examined whether a Batesian mimic and its model are engaged in a coevolutionary arms race. Specifically, I tested whether model populations vary in their phenotypes (i.e. populations should be at different stages of a coevolutionary arms race) and that model populations that experience a high cost of mimics should experience strong selection to evolve phenotypes distinctive from their mimics. I found no evidence for either prediction in *M. fulvius*, suggesting that *M. fulvius* is not engaged in a coevolutionary arms race with its mimics.

My fourth chapter evaluates the utility of camera trapping techniques in artificial prey experiments. I collected observational data from 109 artificial snake prey using video-recording camera traps in three locations in the Americas (Ecuador, Mexico, and USA). I show that observational data collected from camera traps could be useful to artificial prey studies but that the benefit of additional data that videos provide is only worth their financial costs in less than 20% of published artificial prey studies. Thus, camera traps are unlikely to be worth the expense for most artificial prey studies until the cost:benefit ratio decreases.

ACKNOWLEDGEMENTS

I thank the members of my committee for their feedback. I thank B. Allf, M. Brick, R. Devineni, B. Edwin Siurob-Espíndola, C. Iván Hernández-Herrera, D. Joaquin Sánchez-Ochoa, E. Hernández-Martínez, S. Jovita González-Ramos, R. Martin, M. Nuñez, H. Adán Pérez-Mendoza, C. Porter, D. Salazar-Valenzuela, K. Schulze, and J. Vaca Guerrero for their help in the lab and in the field. I thank the following museums for providing access to their specimens: AMNH, ANSP, AUM, BRTC, BYU, CAS, CM, CU, FLMNH, FMNH, GSU, KUNHM, LACM, LSUMZ, MCZ, MPM, MSB, MSM, MVZ, NCSM, OMNH, QCAZ, SBMNH, SDMNH, UA, UAFMC, UCM, UIMNH, UMMZ, UMNH, USNM, UTA, UTEP, and YPM. I thank the Ecuadorian government, Mexican government, and several private landowners and land managers for providing permission to conduct research on their land. I thank the Sigma Xi Grants-In-Aid of Research Program, the Lewis and Clark Fund for Exploration and Research, the Society for the Study of Evolution, the Society for the Study of Amphibians and Reptiles, the Animal Behavior Society, the American Museum of Natural History, the Chicago Herpetological Society, the North Carolina Herpetological Society, the American Society of Naturalists, the NSF Graduate Research Fellowship program, and the Graduate School at the University of North Carolina at Chapel Hill for providing funding to conduct this research.

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CHAPTER 1: RAPID EVOLUTION OF MIMICRY FOLLOWING LOCAL MODEL EXTINCTION[1]

Summary

Batesian mimicry evolves when individuals of a palatable species gain the selective advantage of reduced predation because they resemble a toxic species that predators avoid. Here, we evaluated whether—and in which direction—Batesian mimicry has evolved in a natural population of mimics following extirpation of their model. We specifically asked if the precision of coral snake mimicry has evolved among kingsnakes from a region where coral snakes recently (1960) went locally extinct. We found that these kingsnakes have evolved *more precise* mimicry; in contrast, no such change occurred in a sympatric non-mimetic species or in conspecifics from a region where coral snakes remain abundant. Presumably, more precise mimicry has continued to evolve after model extirpation because relatively few predator generations have passed, and the fitness costs incurred by predators that mistook a deadly coral snake for a kingsnake were historically much greater than those incurred by predators that mistook a kingsnake for a coral snake. Indeed, these results are consistent with prior theoretical and empirical studies, which revealed that only the most precise mimics are favored as their model becomes increasingly rare. Thus, highly noxious models can generate an 'evolutionary momentum' that drives the further evolution of more precise mimicry—even after models go extinct.

[1] This chapter previously appeared as an article in *Biology Letters*. The original citation is as follows: Akcali CK, and Pfennig DW. "Evolution of mimicry following local model extinction," *Biology Letters* 10, no. 6 (June 2014): 20140304.

Introduction

When selection is strong, evolutionary change can occur in natural populations rapidly enough to observe (Hendry and Kinnison 1999). Because selection to avoid being eaten is typically strong (Mallet and Barton 1989), a context in which rapid evolution may readily arise is Batesian mimicry. Batesian mimicry occurs when an edible species (the 'mimic') evolves to resemble a conspicuous, noxious species (the 'model'), thereby gaining protection from predation (Bates 1862; Forbes 2009; Ruxton et al. 2004). The degree of resemblance between mimics and their models is generally sensitive to changes in model abundance (Ruxton et al. 2004; Pfennig et al. 2001); mimetic fidelity can decrease or increase, depending on whether the model becomes relatively more or less abundant, respectively (Harper and Pfennig 2007).

How phenotypic resemblance between mimics and their models changes immediately following model extirpation is unclear, however. Three outcomes are possible. First, mimics may remain *unchanged*. Such an outcome might arise if, for instance, there has not been enough time for mimics to respond to changes in model abundance. Second, *less* precise mimicry may evolve (Brower 1960; Harper and Pfennig 2008). Mimicry may break down following model extirpation because local predators would no longer experience selection to recognize mimics as dangerous Pfennig et al. 2001; Pfennig et al. 2007; Harper and Pfennig 2008). Third, *more* precise mimicry may evolve. Greater mimetic precision may evolve after model extirpation if alternative prey are abundant, and if the fitness costs associated with mistaking a model for a mimic were historically greater than those associated with mistaking a mimic for a model (as might be the case with highly noxious models). Indeed, theoretical (Duncan and Sheppard 1963; Oaten et al. 1975; Sherratt 2002) and empirical studies (Harper and Pfennig 2007; Kikuchi and Pfennig 2010) have shown that only the most precise mimics receive protection from predation when the model

becomes increasingly rare (as would be expected to occur when model extirpation is imminent); thus, selection may continue to favor the evolution of more refined mimicry, even after the model is gone.

Here, we focus on a well-studied mimicry complex to evaluate whether and how Batesian mimicry evolved following extirpation of the model.

Methods

Study System. Nonvenomous scarlet kingsnakes (*Lampropeltis elapsoides*) resemble venomous coral snakes (*Micrurus fulvius*; Figure 1.1). Although both species co-occur in the southeastern U.S., *L. elapsoides* also occurs further north (Figure 1.1). Field experiments have found that natural predators avoid plasticine replicas of *L. elapsoides* in sympatry with *M. fulvius* but not in these northern allopatric regions (Pfennig et al. 2001), verifying that *L. elapsoides* are Batesian mimics of *M. fulvius*. Moreover, even naïve sympatric predators avoid coral snake patterns (Smith 1975).

Historically, *M. fulvius* reached its northernmost limit in the North Carolina Sandhills (Palmer and Braswell 1995), a 3,900 square kilometers area of gently rolling, sand-covered hills characterized by Longleaf Pine savanna (Figure 1.1). Local predators include black bears (*Ursus americanus*), bobcats (*Lynx rufus*), coyotes (*Canis latrans*), foxes (*Vulpes* and *Urocyon* sp.), raccoons (*Procyon lotor*), hawks (*Buteo* sp.), kestrels (*Falco sparverius*), and loggerhead shrikes (*Lanius ludovicianus*).

Micrurus fulvius has always been considered rare in the Sandhills (only five specimens exist in museums; see Supplementary Information), and no recent records exist (Palmer and Braswell 1995). Indeed, no specimens have been collected in the Sandhills since 1960 (Supplementary

Information), despite extensive activity there by herpetologists (Beane et al. 2014). Thus, although the causes are unknown, *M. fulvius* has apparently been extirpated from the Sandhills (or so rare that they are *functionally* extirpated). By contrast, *L. elapsoides* are common in the Sandhills (Palmer and Braswell 1995).

Interestingly, the *L. elapsoides* that most closely resemble *Micrurus* occur in sympatric populations near the sympatry/allopatry border (i.e., 'edge sympatry') (Harper and Pfennig 2007). Field experiments have shown that selection for mimicry is strongest in edge sympatry (Kikuchi and Pfennig 2010). Because the model is rare in edge sympatry (see above), the probability of mistakenly attacking it is low, and predators are therefore more willing to risk attacking imprecise mimics. Consequently, only precise mimics are favored in such edge sympatric regions as the Sandhills (Harper and Pfennig 2007).

Data Collection and Analysis. To determine whether and how mimicry changed over time, we compared 5 pre-extinction *M. fulvius* to 27 post-extirpation *L. elapsoides* from the Sandhills (too few pre-extirpation *L. elapsoides* were available for analysis). These *L. elapsoides* were collected in the 1970s ($N = 5$ individuals), 1980s ($N = 5$), 1990s ($N = 3$), 2000s ($N = 11$), and 2010s ($N = 3$; Supplementary Information). Specimens were photographed using a digital camera (Canon PowerShot SX110; Canon Zoom Lens, 6.0-60.0 mm, 1:2.8-4.3); the width of each ring was measured from digital images using ImageJ 1.46 (Abramoff et al. 2004). We then calculated the proportions of red and black on the mid-dorsum of each snake from its snout to its cloaca. Previous work showed that these characteristics changed the most as the mimetic pattern breaks down in allopatry (Pfennig et al. 2007; Harper and Pfennig 2008) and that these characteristics are targets of predator-mediated selection (Harper and Pfennig 2007; Kikuchi and Pfennig 2010).

We combined the mean proportion of dorsum red and black on mimics and models into a common principle component score. We then subtracted the mean PC1 score for *M. fulvius* from the PC1 score for each individual mimic to calculate a mimic-model dissimilarity score (where a score of zero indicates that *L. elapsoides* and *M. fulvius* were identical in proportion red and black; Supplementary Information). Using JMP 10.0.1, we regressed the dissimilarity score of each *L. elapsoides* against the year it was sampled to determine if resemblance between *L. elapsoides* and *M. fulvius* changed over time (one outlier was omitted from analysis).

Next, we sought to control for the possibility that any change in *L. elapsoides* color pattern might reflect, not predator-mediated selection favoring mimicry, but some other agent of selection (e.g., a change in light environment following recent anthropogenic changes in habitat). We did so in two ways. First, we assessed whether phenotypic changes similar to those observed among *L. elapsoides* from the Sandhills were observed among *L. elapsoides* from the Florida panhandle, where *M. fulvius* remains abundant (see Figure 1.1). This region is similar to the Sandhills in habitat; moreover, the assemblage of predators is similar across regions. Using the methods above, we compared 23 *M. fulvius* and 23 *L. elapsoides* from the Florida panhandle. The *L. elapsoides* were collected in the 1970s ($N = 13$ individuals), 1980s ($N = 1$), 1990s ($N = 2$), 2000s ($N = 7$; Supplementary Information). Second, we assessed whether similar phenotypic changes occurred in corn snakes, *Pantherophis guttatus*, a non-mimetic species found in the Sandhills. Like *L. elapsoides*, *P. guttatus* has red and black on its dorsum, but its pattern is characterized by blotches — not rings. Using the methods above, we sampled 82 *P. guttatus* that were collected in the 1970s ($N = 5$ individuals), 1980s ($N = 14$), 1990s ($N = 18$), 2000s ($N = 41$), and 2010s ($N = 4$; Supplementary Information); these specimens were compared to the 5 *M. fulvius* from the Sandhills (see above).

Results

In 50 years following the apparent extirpation of *M. fulvius*, *L. elapsoides* from the North Carolina Sandhills became more similar to the former in color pattern ($F_{1,26} = 6.997$; $P = 0.014$; Figure 1.2). Moreover, these *L. elapsoides* became less variable in color pattern (Spearman correlation between coefficient of variation in dissimilarity score and decade sampled = -0.8; $N =$ 5 decades; $P = 0.05$ [one-tailed test]). In contrast, *L. elapsoides* from Florida did not change significantly in mimic-model dissimilarity ($F_{1,22} = 1.417$; $P = 0.247$; Figure 1.2) nor did *P. guttatus*, a non-mimetic species from the North Carolina Sandhills ($F_{1,81} = 1.028$; $P = 0.314$; Figure 1.2).

Discussion

Theory predicts that mimicry should break down in the absence of models (Ruxton et al. 2004). Indeed, mimicry in *L. elapsoides* breaks down where it occurs in allopatry with its model (Harper and Pfennig 2008). However, instead of observing an erosion of mimicry following extirpation of *M. fulvius* from the North Carolina Sandhills, we observed rapid evolution of more *precise* mimicry (Figure 1.2). No such pattern was detected among *L. elapsoides* from Florida, where the model has not been extirpated (Figure 1.2), nor among a non-mimetic species from the Sandhills (Figure 1.2).

Two lines of evidence suggest that precise mimicry has *evolved* in the Sandhills. First, snakes were sampled over a 38-year interval, twice the maximum life span of *L. elapsoides* (Isberg 2002). Thus, changes occurred across generations. Second, these changes are unlikely to reflect phenotypic plasticity: there is no evidence of plasticity in *L. elapsoides* coloration (Kikuchi and Pfennig 2012). Thus, our data (Figure 1.2) appear to reflect *evolutionary* change.

This rapid evolution of precise mimicry is consistent with theoretical and empirical studies. Theory predicts that selection for mimetic precision should increase as models become scarcer (Duncan and Sheppard 1963; Oaten et al. 1975; Sherratt 2002), as would likely have occurred in the Sandhills. Additionally, field experiments recently conducted in this population revealed that free-ranging predators only avoid precise (but not imprecise) *L. elapsoides* mimics (Harper and Pfennig 2007; Kikuchi and Pfennig 2010). Thus, predators in the Sandhills continue to exert strong selection for more precise mimicry.

Presumably, the generalist predators in the Sandhills (Palmer and Braswell 1995) likely pay a low cost of passing up a palatable meal by mistaking a mimic for a model. By contrast, because *M. fulvius* are highly venomous (Roze 1996), prior to 1960 (when *M. fulvius* were extirpated), predators likely paid a *high* cost for mistaking a *model* for a *mimic*. This asymmetry in fitness costs explains the strong selection to avoid the model *and* its lookalikes.

Eventually, however, mimicry should break down. How rapidly it does so depends on such factors as the generation times of predators and mimics, the standing variation in coloration among mimics, gene flow between mimics in sympatry vs. allopatry (Harper and Pfennig 2008), and the intensity of selection against mimics.

In sum, our data suggest that, paradoxically, selection imposed on mimics by predators can generate an 'evolutionary momentum' toward more precise mimicry—even after models go extinct.

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Figure 1.1. (a) Nonvenomous scarlet kingsnakes, *Lampropeltis elapsoides*, are Batesian mimics of (b) highly venomous eastern coral snakes, *Micrurus fulvius*. (c) Historically, *M. fulvius* and *L. elapsoides* co-occurred in the North Carolina Sandhills (as shown here). Around 1960, however, *M. fulvius* was apparently extirpated from this region, but not from the Florida panhandle.

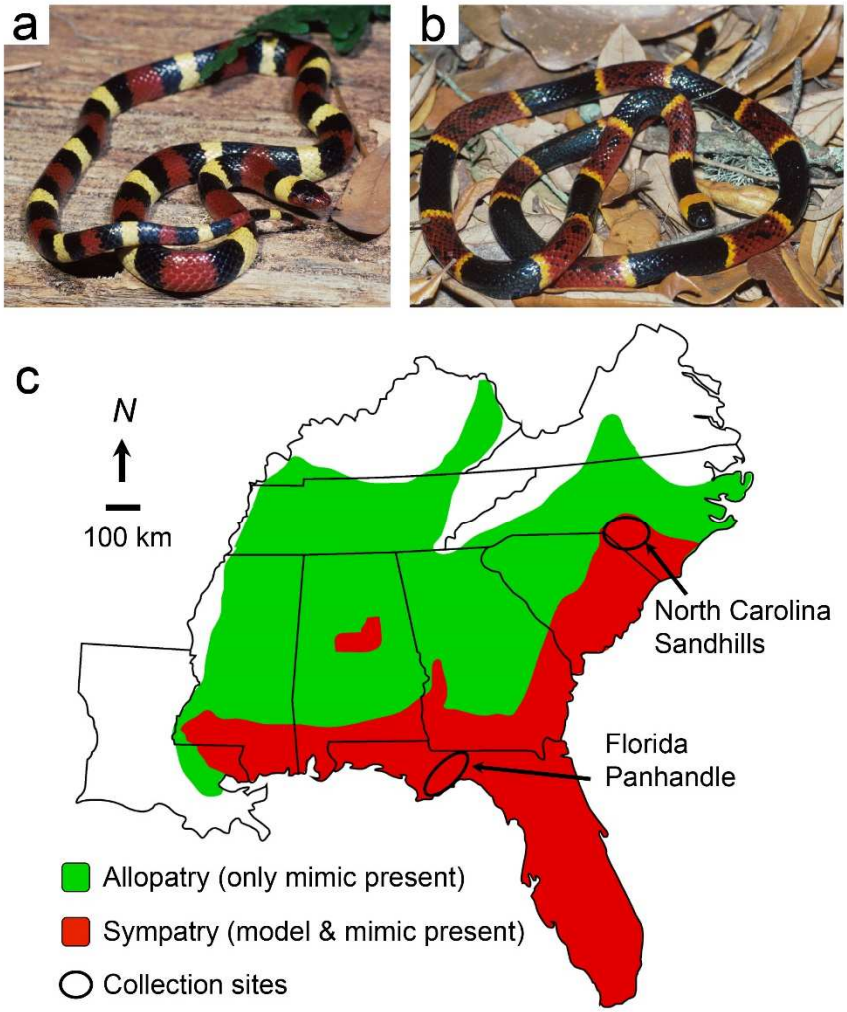
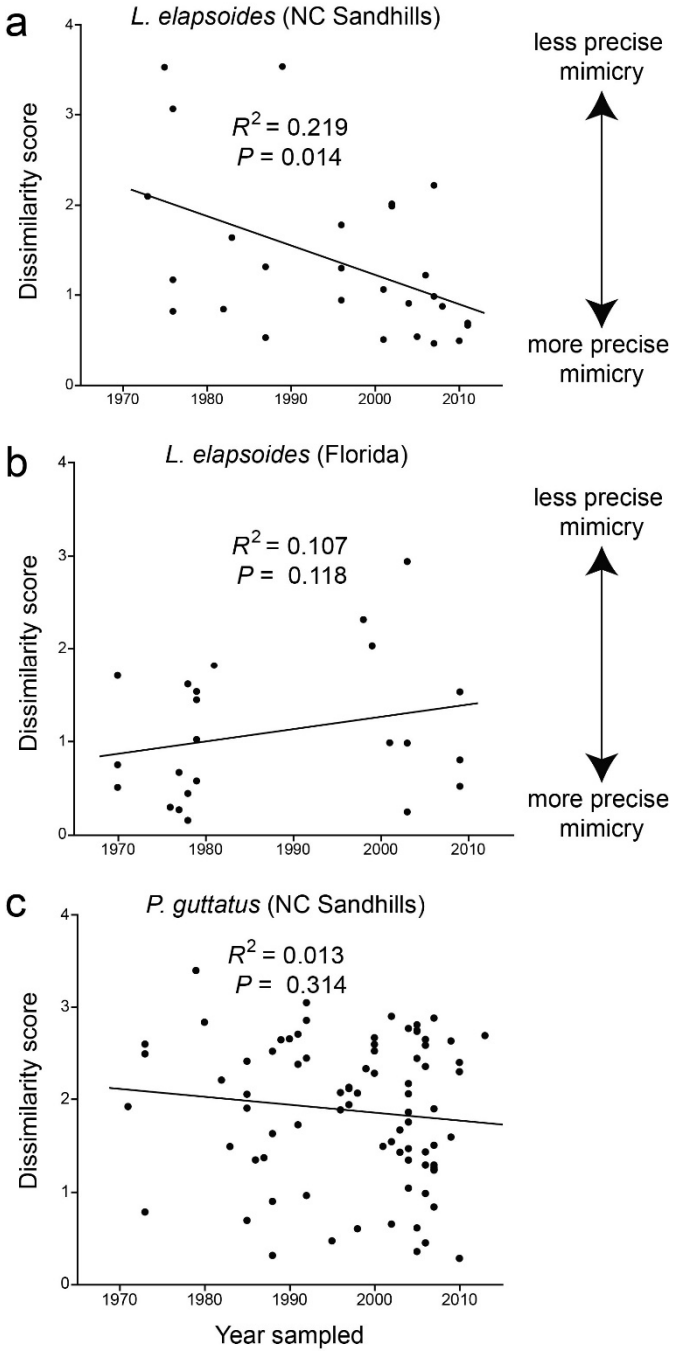


Figure 1.2. (a) Over the past four decades, *L. elapsoides* from the Sandhills (where *M. fulvius* became extirpated around 1960) have become more similar to *M. fulvius*. By contrast, no such trend was found among (b) *L. elapsoides* from the Florida panhandle (where *M. fulvius* remains abundant) or (c) *P. guttatus* (a non-mimetic species) from the Sandhills.



CHAPTER 2: GEOGRAPHIC VARIATION IN MIMETIC PRECISION AMONG DIFFERENT SPECIES OF CORAL SNAKE MIMICS[2]

Summary

Batesian mimicry is widespread, but whether and why different species of mimics vary geographically in resemblance to their model is unclear. We characterized geographic variation in mimetic precision among four Batesian mimics of coral snakes. Each mimic occurs where its model is abundant (i.e., in "deep sympatry"), rare (i.e., at the sympatry/allopatry boundary or "edge sympatry"), and absent (i.e., in allopatry). Geographic variation in mimetic precision was qualitatively different among these mimics. In one mimic, the most precise individuals occurred in edge sympatry; in another, they occurred in deep sympatry; in the third, they occurred in allopatry; and in the fourth, precise mimics were not concentrated anywhere throughout their range. Mimicry was less precise in allopatry than in sympatry in only two mimics. We present several non-mutually exclusive hypotheses for these patterns. Generally, examining geographic variation in mimetic precision—within and among different mimics—offers novel insights into the causes and consequences of mimicry.

[2] This chapter previously appeared as an article in the *Journal of Evolutionary Biology*. The original citation is as follows: Akcali CK, and Pfennig DW. "Geographic variation in mimetic precision among different species of coral snake mimics," *Journal of Evolutionary Biology* 30, no. 7 (July 2017): 1420-1428.

Introduction

Batesian mimicry evolves when a palatable species (the "mimic") gains the selective advantage of reduced predation because it resembles a toxic species (the "model") that predators avoid (Bates 1862; Ruxton et al. 2004). A longstanding assumption of mimicry theory is that natural selection should favor mimics that precisely resemble their models (Ruxton et al. 2004). However, variation in the degree to which mimics resemble their models (i.e., "mimetic precision") is common, and explaining why this variation exists can provide key insights into the causes and consequences of mimicry (reviewed in Kikuchi and Pfennig 2013).

Most studies that have examined variation in mimetic precision have focused on variation between species. For example, colubrid snakes (Pough 1988), hover flies (Penney et al. 2012; Taylor et al. 2013; Taylor et al. 2016a), myrmecomorphic spiders (Pekár et al. 2011), and velvet ants (Wilson et al. 2013) have been shown to vary interspecifically in mimetic precision. Fewer studies have rigorously characterized geographic variation *within* species. Examples include colubrid snakes (Greene and McDiarmid 1981; Harper and Pfennig 2007; Harper and Pfennig 2008), red-backed salamanders (Kraemer et al. 2015), and sedge sprites (Iserbyt et al. 2011).

Although there are several non-mutually exclusive selective reasons for why mimics vary in resemblance to their model (reviewed in Kikuchi and Pfennig 2013), studies of geographic variation in mimetic precision within species have revealed two types of patterns. First, mimetic precision has been shown to vary with the frequency of local models (Harper and Pfennig 2007; Iserbyt et al. 2011). Generally, mimetic precision should increase as the mimic: model ratio increases (Oaten et al. 1975; Sherratt 2002; Harper and Pfennig 2007; Kikuchi and Pfennig 2013). This outcome occurs because in regions where the model is common, predators should experience strong selection to avoid anything that remotely resembles the model, thereby

weakening selection for precise mimicry. In contrast, where the model is rare, selection on predators to avoid the model should be relaxed. Consequently, only precise mimics should receive any protection from predation; thus, selection for precise mimicry is expected to be strong in such regions. Because species tend to be rarer at the edges of their range due to selective or demographic constraints (Geber 2008; Steen and Barrett 2015), mimics that occur at the edge of their model's range (i.e., "edge sympatry") should be more precise than those in the center of their model's range (i.e., "deep sympatry"). This pattern—known as "mimicry on the edge"—has been documented in colubrid snakes (Harper and Pfennig 2007).

Second, the precision of mimics has been shown to decrease in locations where their model is absent; i.e., in allopatry (Harper and Pfennig 2008; Ries and Mullen 2008). Although longstanding theory predicts that mimics should only occur in regions inhabited by their model (Wallace 1870; Ruxton et al. 2004), many species of mimics violate this prediction and also occupy regions where their model is absent (Pfennig and Mullen 2010). Although mimics might benefit from being conspicuous in the absence of models if, for instance, predators innately avoid such signals, mimics that occur in such locations are generally expected to experience selection against mimetic phenotypes because such phenotypes are typically aposematic and thus conspicuous to potential predators (Ruxton et al. 2004). Therefore, mimics are expected to resemble their models less precisely in allopatry, with the degradation of the mimetic phenotype increasing as the distance from the sympatry/allopatry boundary increases. This pattern—dubbed "mimetic breakdown"—has been reported in colubrid snakes (Harper and Pfennig 2008) and butterflies (Ries and Mullen 2008).

How general mimicry on the edge and mimetic breakdown are among mimicry complexes—as well as the underlying causes of such geographic patterns in mimetic precision—

is unknown. Here, we characterize geographic variation in mimetic precision among four Batesian mimics of coral snakes. We address three questions. First, how common is mimicry on the edge? Second, how widespread is mimetic breakdown? Finally, what are the possible causes of any such geographic variation in mimetic precision?

Methods

Study System and Geographic Sampling

Several species of harmless colubrid snakes mimic highly venomous New World coral snakes. We focused on four such mimics (Figure 2.1). In the southeastern U.S., scarlet kingsnakes (*Lampropeltis elapsoides*) and scarlet snakes (*Cemophora coccinea*) mimic eastern coral snakes (*Micrurus fulvius*). In the southcentral U.S., Western milk snakes (*Lampropeltis gentilis*), *C. coccinea*, and the recently described Texas scarlet snake (*Cemophora lineri*; Weinell and Austin 2017) mimic Texas coral snakes (*Micrurus tener*). All of the *C. coccinea* and *C. lineri* that co-occurred with *M. tener* were considered to be one mimic (Western *C. coccinea*) since the two species do not differ in any mimetic traits and share the same model. All of the *C. coccinea* that co-occurred with *M. fulvius* were called Eastern *C. coccinea*. All four mimics (*L. elapsoides*, Eastern *C. coccinea*, *L. gentilis*, and Western *C. coccinea*) occur in regions where models are abundant (deep sympatry), rare (edge sympatry), and absent (allopatry).

We sampled 2,880 snakes, nearly all of which were from museum collections: 2,053 mimics (841 *L. elapsoides*, 963 Eastern *C. coccinea*, 130 *L. gentilis*, and 119 Western *C. coccinea*) and 827 models (523 *M. fulvius* and 304 *M. tener*). Our sampling regime included most of the geographic range of each model and mimic species within the U.S.

Quantifying Mimetic Precision

Specimens were photographed using a digital camera (Canon PowerShot SX130 IS; Canon Zoom Lens, 6.0-60.0 mm, 1:2.8-4.3); the length of each ring was measured from digital images using ImageJ v. 1.46 (Abramoff et al. 2004). Digital measurements of photographs is one of the most consistent and accurate methods to make length measurements on live and preserved snakes (Astley et al. 2017). We then calculated the proportions of red and black on the mid-dorsum of each snake, from snout to cloaca. We limited our analysis to red and black because these are the predominant colors on both models and mimics and because including all three colors (black, red, and yellow/white) would remove the independence of the characteristics. In addition, previous work has revealed that the proportions of red and black change the most as the mimetic pattern breaks down in allopatry (Pfennig et al. 2007; Harper and Pfennig 2008), and that these characteristics are targets of predator-mediated selection in the U.S. (Harper and Pfennig 2007; Kikuchi and Pfennig 2010a; Kikuchi and Pfennig 2010b; Pfennig et al. 2015).

Mimetic precision was assessed using previously described methods (Akcali and Pfennig 2014; Figure 2.2; see Figure 2.3 and Figure 2.4 for additional sample photographs). Specifically, we combined the proportion of dorsum red and black on mimics and models into a common principal component (PC1) using JMP v. 10.0.1. A "dissimilarity score" (D) was then computed by taking the absolute value of the difference between the PC1 scores of mimics and models. Thus, higher values of D correspond to less precise mimicry, whereas lower values of D correspond to more precise mimicry.

Assessing Mimicry on the Edge

Geographic variation was visualized by constructing phenotypic rasters for each mimic and model using local inverse distance-weighted (IDW) interpolation of PC1 scores of georeferenced mimic and model specimens. A critical assumption of IDW is that the interpolated traits are spatially autocorrelated (Cromley 1992). To test for spatial autocorrelation within each mimic and model in PC1, we computed Moran's *I*, a commonly used measure of spatial autocorrelation in ecology (Legendre and Fortin 1989). We calculated values of Moran's *I* at varying distances at a 50-km interval up to the highest possible spatial extent. We tested for the significance of the Moran's *I* statistic for PC1 locally (at each distance class) as well as globally (at all distance classes) after Bonferroni correction. Spatial outliers were removed prior to conducting IDW analyses in mimics or models that did not meet the assumption of global spatial autocorrelation in PC1 when they were included.

Phenotypic rasters for each mimic and model included all specimens from sympatry, and additionally, for mimics, all allopatric specimens within 30 km of the sympatry/allopatry boundary (Figure 2.5; Figure 2.6). All IDW analyses were conducted at a 1-km resolution, a power function of one, and a fixed radius of inference of 150 km, as spatial autocorrelation tended to be low beyond 150 km (Figure 2.7). Although parameter selection can affect the outcome of IDW analyses, most species were sampled thoroughly enough such that estimated surfaces were robust to major changes in parameter values. To visualize geographic patterns of mimic-model resemblance, we took the absolute value of the difference between mimic and model rasters. The resulting raster was then clipped to a geographic range polygon of sympatry. Geographic range data for each mimic and model species (except for *L. gentilis*) were downloaded from the IUCN Red List of Threatened Species (www.iucnredlist.org, accessed on 1

July 2015). Geographic range polygons were modified by hand according to our own knowledge of species distributions prior to being used in analyses. The geographic range polygon for *L. gentilis* was constructed by hand based on the distribution of vouchered specimens (Werler and Dixon 2000). All analyses were performed in ArcMap 10.1.

To statistically determine whether mimics exhibit mimicry on the edge, we extracted interpolated PC1 values of models at the location of each mimic specimen and used these values to compute D scores for each mimic specimen. We then tested for differences in D between deep sympatric (D_{DS}) and edge sympatric (D_{ES}) mimics at 50-km intervals from the sympatry/allopatry boundary using two-tailed *t*-tests. The sympatry/allopatry boundary was estimated from published accounts (Palmer and Braswell 1995; Mount 1975; Jensen et al. 2008; Trauth et al. 2004; Werler and Dixon 2000) supplemented by recent vouchered records from the VertNet database (http://portal.vertnet.org/search) that had accurate locality information. Because D_{ES} was subtracted from D_{DS}, positive values indicate that deep sympatric mimics are more dissimilar (and thus more imprecise) than edge sympatric mimics; negative values indicate that edge sympatric mimics are more precise than deep sympatric mimics; and values close to 0 indicate that edge sympatric and deep sympatric mimics do not differ in D. The total number of distance classes used varied among mimics and depended on the spatial extent of the sampled specimens. We plotted differences in D calculated at each distance class against distance from the sympatry/allopatry boundary to visualize how D changes in sympatry. Thus, mimics with consistently positive differences in D show "mimicry on the edge," while mimics with consistently negative differences in D show the opposite pattern—imprecise mimics on the edge.

Assessing Mimetic Breakdown

To determine whether mimicry breaks down in allopatry, we compared the D of sympatric mimics (*L. elapsoides*, $N = 699$; Eastern *C. coccinea*, $N = 663$; *L. gentilis*, $N = 78$; Western *C. coccinea*, $N = 73$) and allopatric mimics (*L. elapsoides*, $N = 142$; Eastern *C. coccinea*, $N = 300$; *L. gentilis*, $N = 52$; Western *C. coccinea*, $N = 46$) to their models (*M. fulvius*, $N = 525$; *M. tener*, $N = 304$) using two-tailed *t*-tests. Mimicry was considered to break down in allopatry if the D scores of allopatric mimics were significantly higher than the D scores of sympatric mimics. To determine whether mimicry degrades with increasing distance into allopatry, we used regression analyses to assess the relationship between the mimetic precision of each snake and distance from the sympatry/allopatry boundary. D scores for sympatric mimics and allopatric mimics <30 km from the sympatry/allopatry boundary were generated using the methods described in the previous section. D scores for allopatric mimics >30 km from the sympatry/allopatry boundary were generated by comparing the PC1 value of each mimic specimen to the mean PC1 value of their model.

Results

Assessing Mimicry on the Edge

PC1 for all snake species met the assumption of spatial autocorrelation at local and global scales (Figure 2.7). Spatial outliers were removed from Western *C. coccinea* prior to conducting IDW analyses to meet spatial autocorrelation assumptions (Figure 2.7). Patterns of geographic variation in mimetic precision in sympatry were highly variable among coral snake mimic species (Figure 2.8). The difference between D_{DS} and D_{ES} was positive in *Lampropeltis elapsoides*, indicating that this species exhibited mimicry on the edge (Figure 2.8; Figure 2.9). In

contrast, the difference between D_{DS} and D_{ES} was negative in Eastern *C. coccinea* (Figure 2.9). Thus, Eastern *C. coccinea* did not exhibit mimicry on the edge; instead, the best mimics of Eastern *C. coccinea* were located deep in sympatry (Figure 2.8). On the other hand, *L. gentilis* and Western *C. coccinea* did not vary throughout their geographic ranges in sympatry with their model: differences in D were not significant at any distance (Figure 2.8, Figure 2.9).

Assessing Mimetic Breakdown

Patterns of mimicry between sympatry and allopatry were also highly variable among coral snake mimic species (Figure 2.10, Figure 2.11). *Lampropeltis elapsoides* and Eastern *C. coccinea* both exhibited mimetic breakdown: mimics in allopatry were more imprecise than those in sympatry (Figure 2.11). However, *L. elapsoides* and Eastern *C. coccinea* differ in how their mimetic pattern breaks down over space. In *L. elapsoides*, D is spatially bimodal: D is high deep in sympatry (in southern Florida), low close to the sympatry/allopatry boundary, and then becomes high again in allopatry ($F_{2,840} = 23.047$; $P < 0.0001$; Figure 2.10). In Eastern *C. coccinea*, D gradually increases from sympatry to allopatry ($F_{1,962} = 43.792$; $P < 0.0001$; Figure 2.10). *Lampropeltis gentilis* tended to be more precise in allopatry than in sympatry (Figure 2.11), with D gradually decreasing across the sympatry/allopatry boundary ($F_{1,129} = 3.266$; $P = 0.0731$; Figure 2.10). Western *C. coccinea* mimics did not differ in D between sympatry and allopatry (Figure 2.11) and did not exhibit any spatial variation across the sympatry/allopatry boundary ($F_{1,118} = 0.635$; $P = 0.4272$; Figure 2.10).

Discussion

Different species of coral snake mimics exhibited different patterns of geographic variation in mimetic precision. Mimicry in *L. elapsoides* was most precise at the edge (Figure 2.8, Figure 2.9) and broke down sharply in allopatry (Figure 2.10, Figure 2.1); mimicry in Eastern *C. coccinea* was most precise in deep sympatry (Figure 2.8, Figure 2.9) and broke down gradually across the sympatry/allopatry boundary (Figure 2.10, Figure 2.11); mimicry in *L. gentilis* did not vary in sympatry (Figure 2.8, Figure 2.9) and became more precise across the sympatry/allopatry boundary (Figure 2.10, Figure 2.11); and mimicry in Western *C. coccinea* was mostly invariant throughout its range (Figure 2.8, Figure 2.9, Figure 2.10, Figure 2.11).

Such variable patterns suggest that the causes of geographic variation in mimetic precision likely differ between species. This finding has important implications for mimicry studies. If geographic patterns of mimicry are variable among mimic species more generally, then the field of mimicry would benefit from case studies that conduct more empirical tests of alternative mechanisms that could contribute to explaining such variation (e.g., Harper and Pfennig 2007; Harper and Pfennig 2008).

One question to consider is whether these differences in geographic variation in mimicry between species could be explained by the fact that the genetic control of mimetic color pattern varies among the mimics in our study. Currently, nothing is known of the genetic control of mimetic color pattern in any of our study species. However, genetic crossing studies of several non-mimetic colubrid snakes, such as kingsnakes (Zweifel 1981), gopher snakes (Bechtel and Whitecar 1983), rat snakes (Bechtel and Bechtel 1985), corn snakes (Bechtel and Bechtel 1989, and garter snakes (King 2003) have revealed two major findings: (1) the inheritance of color and pattern traits tends to be under simple genetic control, and (2) the genetic control of color and

pattern are independent, but intimately connected, suggesting that loci for color and pattern traits are genetically linked or in linkage disequilibrium. However, a recent study of a polymorphic coral snake mimic (*Sonora semiannulata*; Davis Rabosky et al. 2016a) has found no evidence that loci for red and black pigmentation are linked or are in linkage disequilibrium. Given that color and pattern traits are under simple genetic control in a wide variety of non-mimetic and mimetic colubrid snakes, we consider it unlikely that species-specific variation in the genetic control of color and pattern explains differences in geographic variation in mimicry between the mimic species in our study.

Another important question that follows from our study is how generalized these variable geographic patterns in mimetic precision within sympatry may be across New World coral snake mimics—especially in the tropics, where mimic, model, and color pattern diversity are higher (Davis Rabosky et al. 2016). It is possible that the patterns of geographic variation in mimetic precision of *L. elapsoides*, *L. gentilis*, and *Cemophora* are atypical among New World coral snake mimics because these mimics occur at the northern edges of the distribution of coral snakes, whereas the majority of coral snake mimics that occur in the tropics co-occur with multiple models and are exposed to more diverse predator communities. However, many species of closely related, mimetic *Lampropeltis* occur throughout the New World (Ruane et al. 2014), yet all of these species share the same color pattern with the mimic species in our study and quantitatively vary in the same traits (Akcali, C.K. unpublished data). Additionally, New World coral snake mimics (as well as their models) from Central and South America show quantitative variation in several color pattern traits between and within different geographic areas—even in species that are known to exhibit discrete morphs in different populations as well as among polymorphic populations (Akcali, C.K. et al. unpublished manuscript). Thus, the variable

geographic patterns in mimetic precision observed *within* sympatry among the northerly species in our study likely represent a common condition among coral snake mimics generally. Whether the patterns of variation *between* sympatry and allopatry observed in our study are common among coral snake mimics generally cannot be answered since regions of complete allopatry with coral snakes in the tropics are nonexistent.

How general our results will prove to be beyond coral snake mimics is uncertain. Most studies of variation in mimetic precision in insects have examined variation between species (Penney et al. 2012; Taylor et al. 2013; Wilson et al. 2013; Taylor et al. 2016). The few studies that have examined variation in mimetic precision within species in insects (Iserbyt et al. 2011) and other vertebrates (Kraemer et al. 2015) did not examine multiple mimic species. Thus, more analyses of intraspecific variation in mimetic precision need to be conducted in more species in other groups to determine how common such variation is in nature. Indeed, studies of more diverse taxa would also help clarify whether hotspots and coldspots *generally* occur in the geographical mosaic of coevolution between Batesian mimics and their models (sensu Thompson 2005), as we found in our study (Figure 2.8).

Our results suggest several more intriguing questions deserving of future study. For example, why are mimics not always most precise in regions where models are rare, such as at range edges, as predicted by theory (Harper and Pfennig 2007; Iserbyt et al. 2011; Kikuchi and Pfennig 2013)? Eastern *C. coccinea* were most precise in deepest sympatry, which is the opposite pattern found in its co-mimic, *L. elapsoides* (compare Figure 2.8). A possible explanation is that these two species affect each other's mimicry evolution via character displacement (*sensu* Brown and Wilson 1956). Phenotypically similar species, such as co-mimics for example (Elias et al. 2008), often compete for resources, successful reproduction, or

both, and, as an adaptive response to minimize such costly interactions, selection might cause interacting species to diverge—in both phenotype and habitat (Pfennig and Pfennig 2012). Such divergence between co-mimics may thereby result in the evolution of imprecise mimicry in one of the mimic species that loses the competition for the mimetic "niche" (e.g., the microhabitat where the model occurs; Pfennig and Kikuchi 2012).

Alternatively, *C. coccinea* might fail to show mimicry on the edge if there is a selective trade-off between mimicry and thermoregulation: mimics that occur at higher latitudes might need more black coloration to achieve optimal body temperatures more rapidly (as in hoverflies; see Taylor et al. 2016b). Contrary to this expectation, however, all snake species—both mimics and models—either had less black on their patterns at higher latitudes or did not vary significantly with latitude (Figure 2.12). Additionally, although mimics with more black did tend to resemble their models more poorly, mimics with little black on their patterns were just as poor mimics as those with more black on their patterns (Figure 2.13).

Lastly, mimics might fail to show mimicry on the edge if selection on smaller mimics is relaxed as a consequence of their lower nutritional content. In other words, there might be a relationship between mimic-model dissimilarity (D) and the nutritional content (i.e., body size) of mimics (as in hoverflies; see Penney et al. 2012) that could obscure the effect of model abundance. However, none of the mimic species are known to vary ontogenetically in their coloration, and there is no evidence of plasticity in their coloration (Kikuchi and Pfennig 2012). Moreover, there was no significant relationship between body size and D in any of the mimic species (Figure 2.14).

Two additional, related questions are: why are *L. gentilis* more precise in allopatry than in sympatry, and why does mimicry fail to break down in Western *C. coccinea*? A possible answer

to both questions is that selection might maintain—and even favor—precise allopatric mimicry if predators migrate between sympatric and allopatric regions (Poulton 1909). For example, migratory birds that exhibit innate (Smith 1975; Smith 1977) or learned avoidance of coral snakes (e.g., due to their co-occurrence with coral snakes on their overwintering grounds) might subsequently avoid conspicuous lookalikes in areas where coral snakes do not occur (i.e., allopatry). Indeed, the diversity and abundance of raptors that migrate from sympatry with coral snakes into allopatry appears to be higher west of the Mississippi River than in the southeastern U.S. (Sauer et al. 2014). Additionally, gene flow among mimics from sympatry into allopatry might also maintain precise allopatric mimicry. For example, gene flow has been shown to be one of the major reasons why mimics of *L. elapsoides* persist in allopatry, despite selection against mimicry in allopatry (Pfennig et al. 2001; Pfennig et al. 2007; Harper and Pfennig 2008).

Regardless of why mimicry fails to break down in allopatry, these findings have important implications for Batesian mimicry's role in local adaptation and, potentially, speciation (Pfennig and Mullen 2010; Pfennig et al. 2015; Davis Rabosky et al. 2016b). Generally, populations of mimics that occur in sympatry with their model versus those in allopatry are expected to experience contrasting selective pressures (Pfennig et al. 2001). In addition, mimics could also experience contrasting selective pressures if mimics occur on different background substrates in sympatry and allopatry (e.g., *L. elapsoides* would primarily be viewed against deciduous leaf litter by predators in allopatry and against pine needles in sympatry). If these opposing selective pressures lead to the evolution of different locally adapted phenotypes (i.e., mimetic phenotypes in sympatry and non-mimetic phenotypes in allopatry; Harper and Pfennig 2008; Ries and Mullen 2008), then selection might further favor the evolution of reproductive isolating barriers—and, possibly, speciation—between such

populations (Nosil 2012). Indeed, at least two isolating barriers appear to have evolved between sympatric and allopatric populations of *L. elapsoides* (Pfennig et al. 2015). However, if mimicry does not generally break down in allopatry, then Batesian mimicry might not play an important role in promoting local adaptation and speciation. Additional studies are needed to determine how common mimetic breakdown is.

In sum, characterizing variation in mimicry and explaining why such variation exists is critically important for understanding how the complex adaptation of mimicry evolves. Our data suggest that the causes of geographic variation in mimicry differ among mimic species. More detailed case studies of single mimicry complexes are needed to evaluate whether there are any general explanations for the causes of variation in the precision of mimicry in nature.

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Figure Legends

Figure 2.1. Coral snake mimics and coral snakes of the southeastern and southcentral United States. In the southeastern U.S., scarlet kingsnakes (*Lampropeltis elapsoides*) and scarlet snakes (*Cemophora coccinea*) mimic eastern coral snakes (*Micrurus fulvius*). In the southcentral U.S., Western milk snakes (*Lampropeltis gentilis*), scarlet snakes (*C. coccinea*), and Texas scarlet snakes (*Cemophora lineri*) mimic Texas coral snakes (*Micrurus tener*).

Figure 2.2. Sample measurements and dissimilarity (D) calculations in two *L. elapsoides* specimens. After taking dorsal photographs of each specimen, a segmented line was traced along the most mid-dorsal scale row from the snout to the cloaca in ImageJ. Specifically, the line began at the tip of the rostral scale and was traced in between the internasals and prefrontals, down the middle of the frontal, in between the parietal scales, and then continued along the dorsal scale row that began in between the parietal scales until the cloaca was reached. The first image shows how the line would begin to be traced from the tip of the snout to the first several scales along the dorsal scale row that began in between the parietal scales on a recently dead specimen before it was preserved (Step 1). The next two images show how the line would be traced along the rest of the body to the cloaca (which was marked with a pin unless the reproductive organs were everted) and then to the tail to measure total length in two preserved specimens (Step 2). The proportions of red and black on each mimic and model specimen were then used to calculate PC1 values for each specimen (Step 3). PC1 values estimated the relative amount of red and black on the dorsum of each snake. Dissimilarity values for each mimic used in the analysis of sympatric mimics were then calculated using PC1 values for models that were extracted from interpolated rasters of model phenotypes at the location of each mimic specimen.

Figure 2.3. Photographs of *L. elapsoides* (a), *M. fulvius* (b), and Eastern *C. coccinea* (c) specimens that illustrate the range of variation in phenotype in each species.

Figure 2.4. Photographs of *L. gentilis* (a), *M. tener* (b), and Western *C. coccinea* (c) specimens that illustrate the range of variation in phenotype in each species.

Figure 2.5. Geographic variation in the proportion of red and black and sympatric specimens (specimens in sympatry and within 30 km of the sympatry-allopatry boundary) that were sampled in *L. elapsoides* (a), *M. fulvius* (b), and Eastern *C. coccinea* (c).

Figure 2.6. Geographic variation in the proportion of red and black and sympatric specimens (specimens in sympatry and within 30 km of the sympatry-allopatry boundary) that were sampled in *L. gentilis* (a), *M. tener* (b), and Western *C. coccinea* (c).

Figure 2.1

Mimics

L. elapsoides

C. coccinea
(East)

L. gentilis

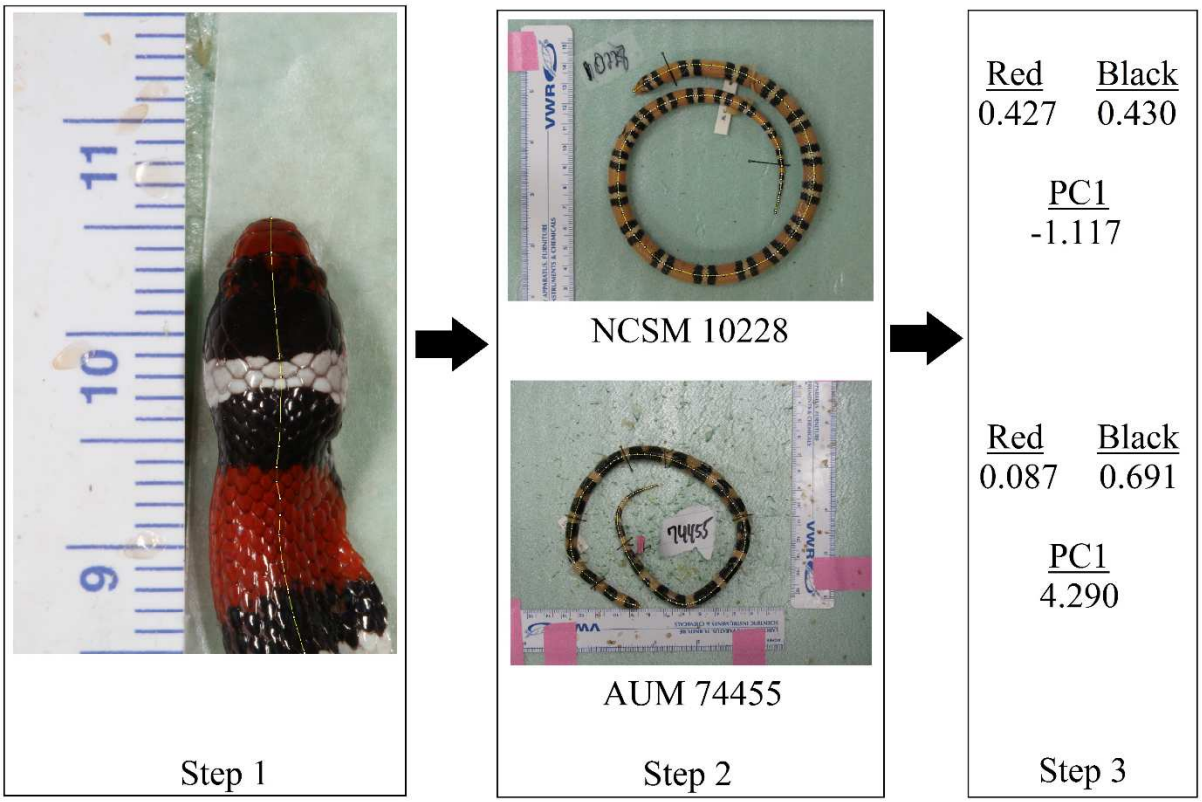
C. coccinea
(West)

Models

M. fulvius

M. tener

Figure 2.2



Dissimilarity Calculation:

NCSM 10228

Dissimilarity (D) = $|PC1_{mimic} - PC1_{model}| = |-1.117 - 0.222| = 1.339$

AUM 74455

Dissimilarity (D) = $|PC1_{mimic} - PC1_{model}| = |4.290 - 1.097| = 3.193$

Figure 2.3

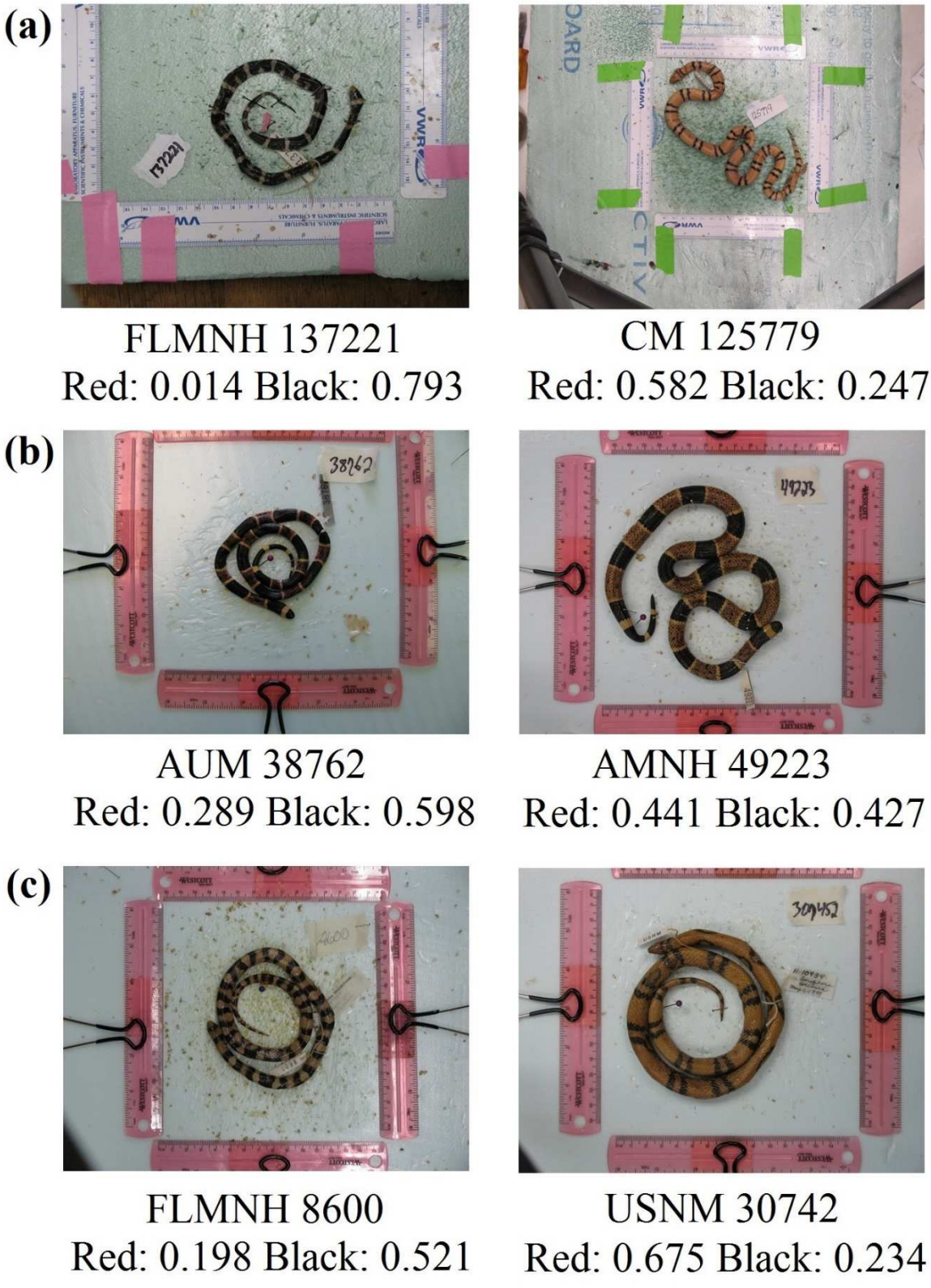

(a)
137221
FLMNH 137221
Red: 0.014 Black: 0.793
CM 125779
Red: 0.582 Black: 0.247
(b)
38762
AUM 38762
Red: 0.289 Black: 0.598
49223
AMNH 49223
Red: 0.441 Black: 0.427
(c)
8600
FLMNH 8600
Red: 0.198 Black: 0.521
30742
USNM 30742
Red: 0.675 Black: 0.234

Figure 2.4

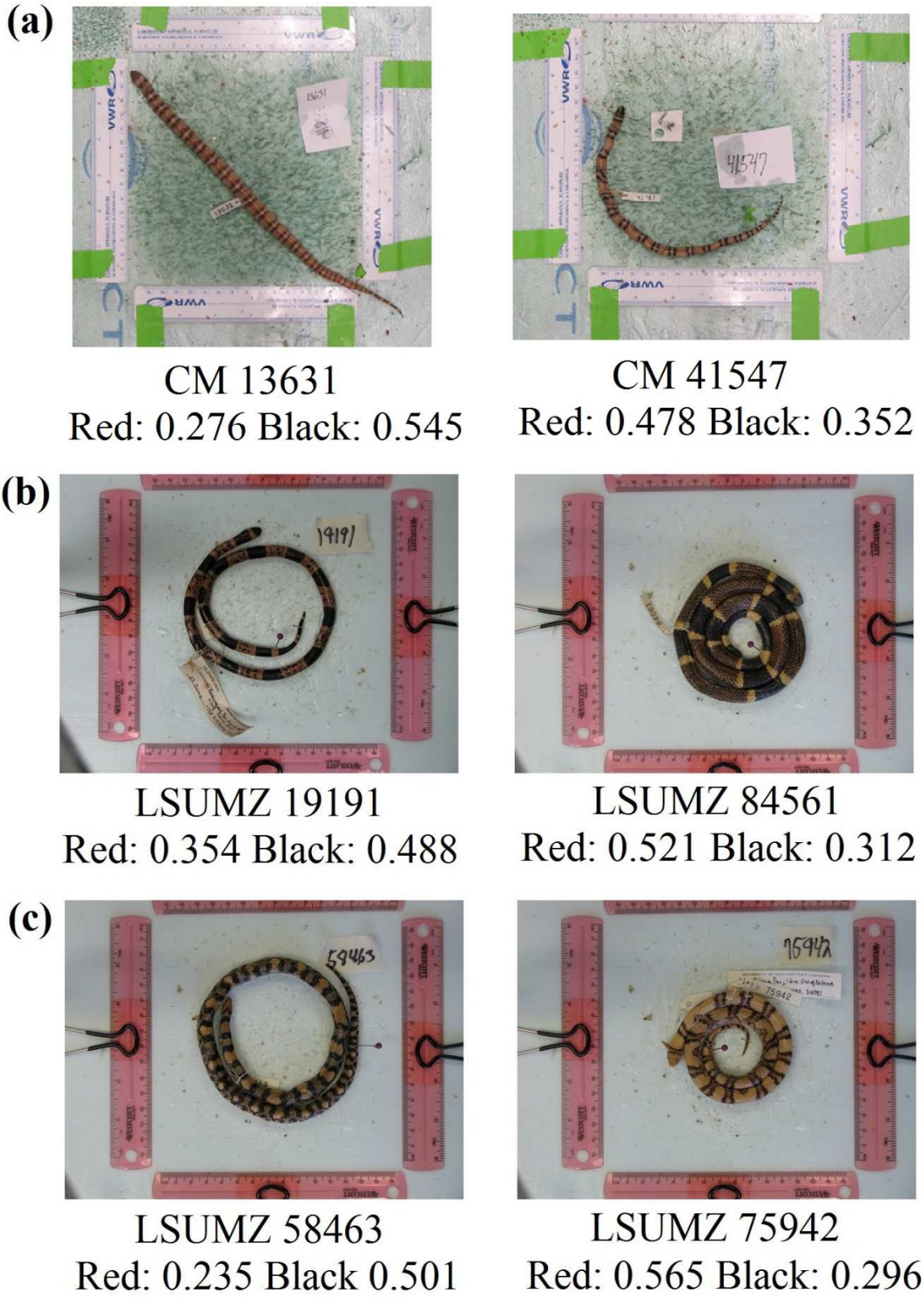

(a)
CM 13631
Red: 0.276 Black: 0.545
CM 41547
Red: 0.478 Black: 0.352
(b)
LSUMZ 19191
Red: 0.354 Black: 0.488
LSUMZ 84561
Red: 0.521 Black: 0.312
(c)
LSUMZ 58463
Red: 0.235 Black 0.501
LSUMZ 75942
Red: 0.565 Black: 0.296

Figure 2.5

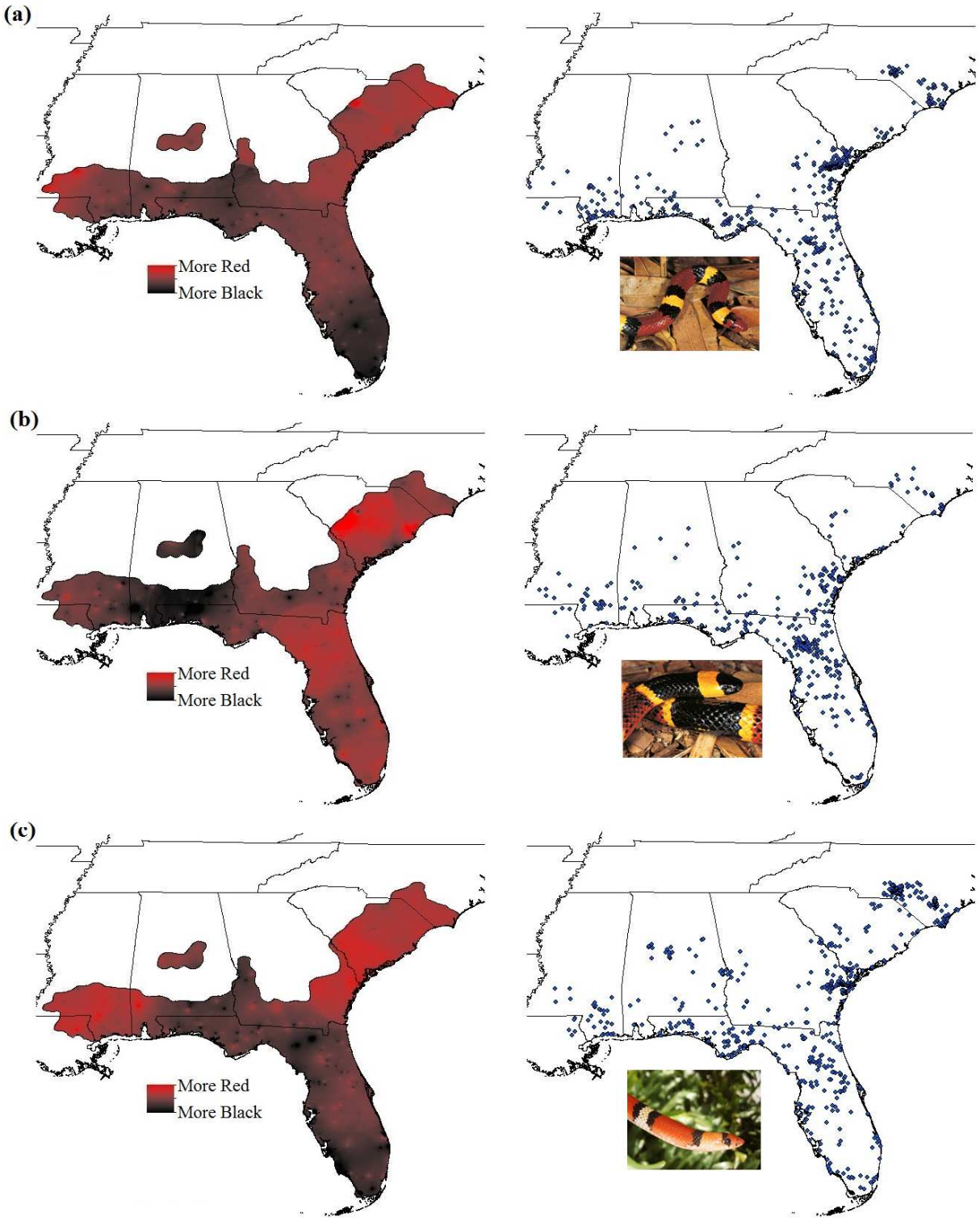

(a)
More Red
More Black
(b)
More Red
More Black
(c)
More Red
More Black

Figure 2.6

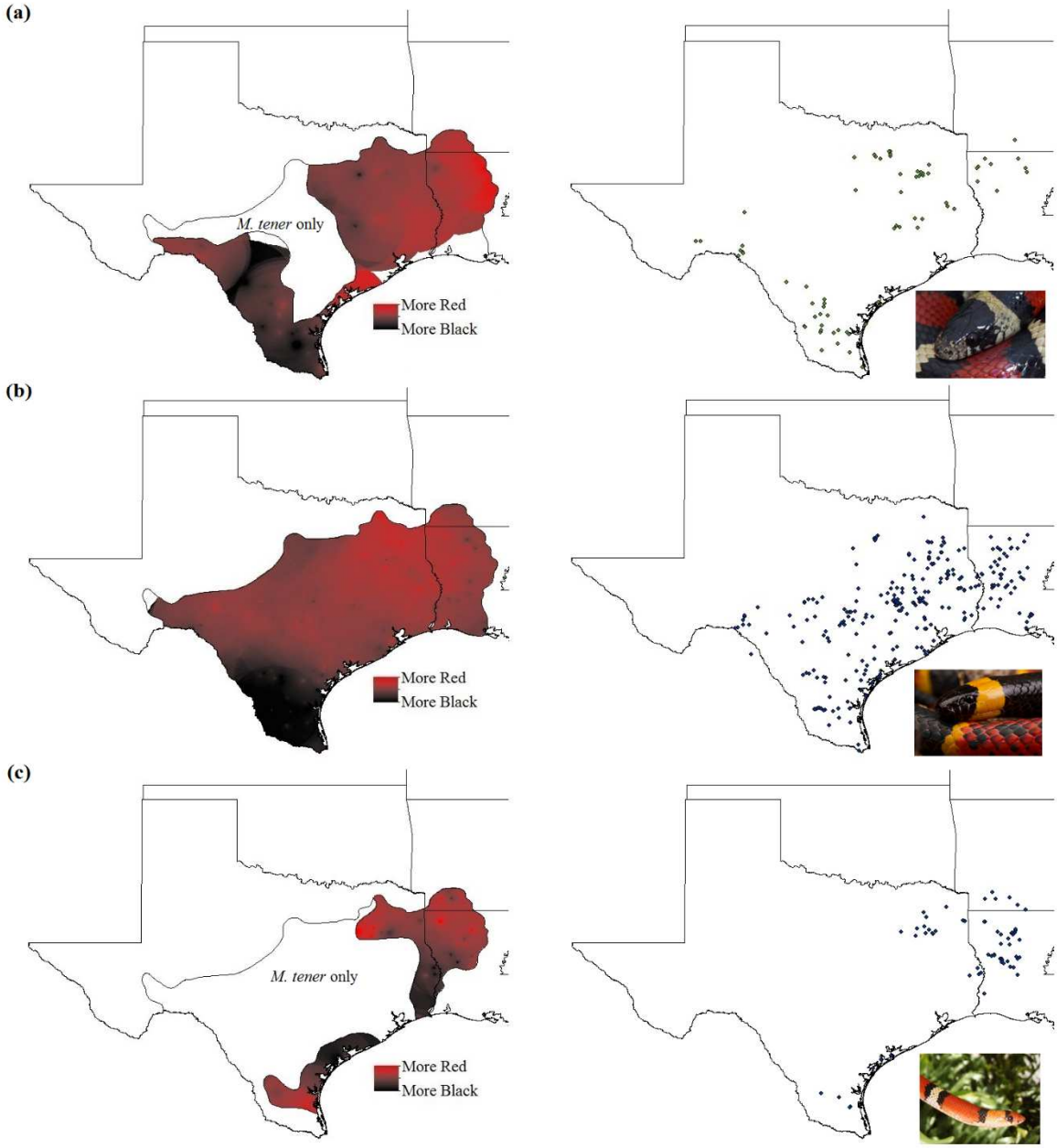

(a)
M. tener only
More Red
More Black
(b)
More Red
More Black
(c)
M. tener only
More Red
More Black

Figure 2.7. Moran's *I* spatial correlograms for PC1 in *L. elapsoides*, Eastern *C. coccinea*, *L. gentilis*, Western *C. coccinea*, *M. fulvius*, and *M. tener*. Significant coefficients (α = 0.05) are indicated by filled symbols (non-significant coefficients are indicated by open symbols). The spatial correlogram for each mimic and model is globally significant after a Bonferroni correction (α' = 0.05/k, where k is the number of distance classes). Three spatial outliers needed to be removed from the Western *C. coccinea* dataset to meet spatial autocorrelation assumptions.

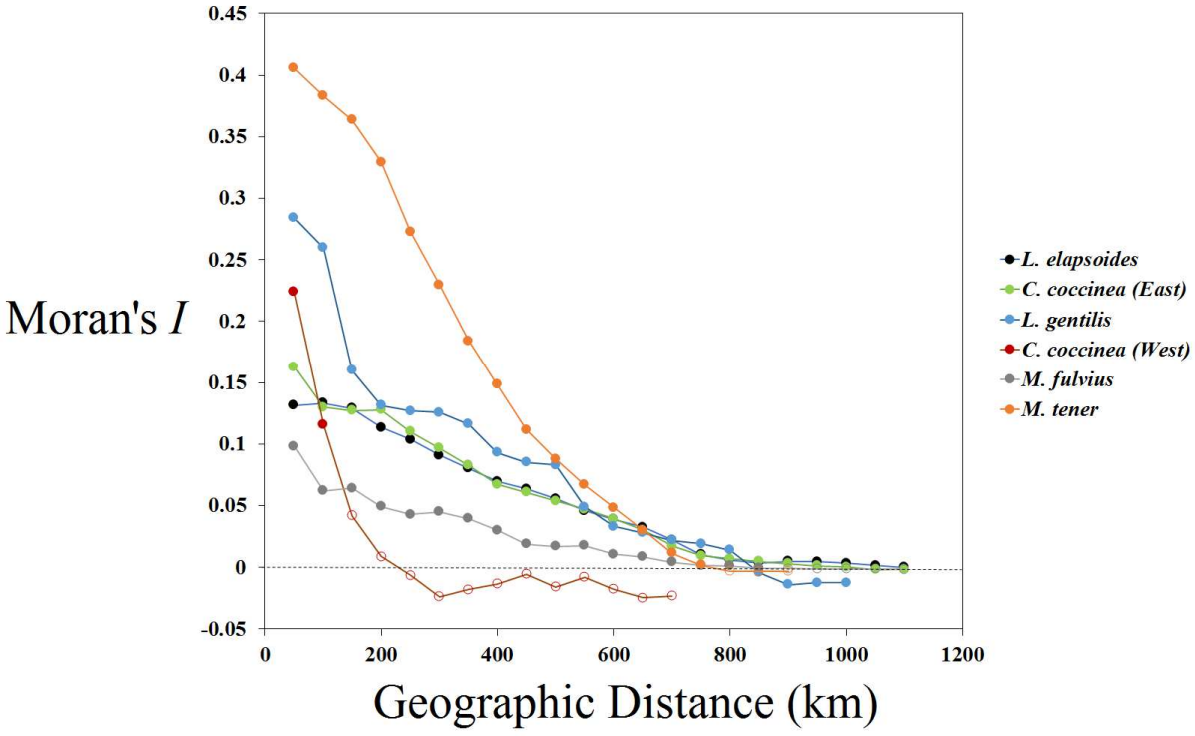


Figure 2.8. Geographic variation in the resemblance of coral snake mimics (a, *L. elapsoides*; b, Eastern *C. coccinea*; c, *L. gentilis*; d, Western *C. coccinea*) to their models (a, b, *M. fulvius*; c, d, *M. tener*) in sympatry. Warmer (red) values correspond to low dissimilarity (i.e., precise mimicry); cooler (blue) values correspond to high dissimilarity (i.e., imprecise mimicry).

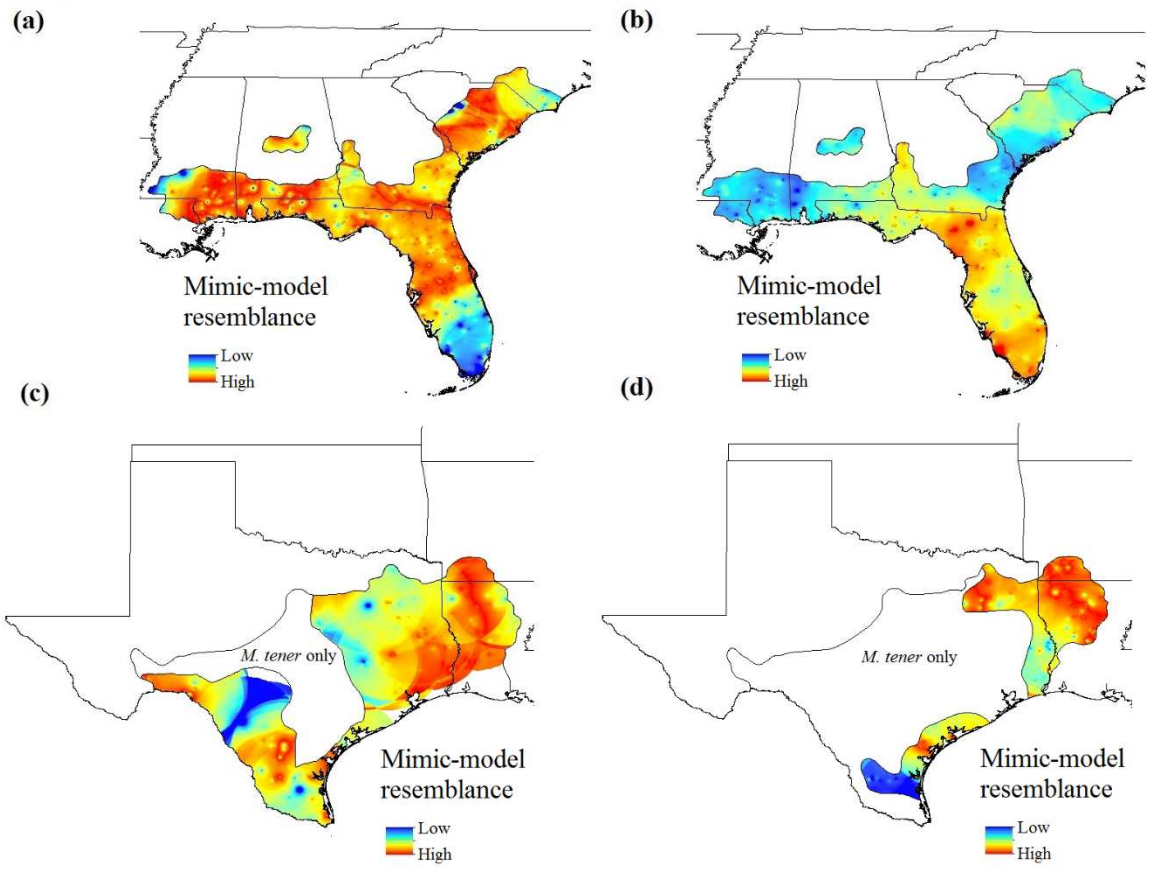


Figure 2.9. Difference in dissimilarity between deep sympatry (DS) and edge sympatry (ES) mimics of *L. elapsoides*, Eastern *C. coccinea*, *L. gentilis*, and Western *C. coccinea* at varying distances from the sympatry/allopatry boundary. Significant differences (two-tailed *t*-tests; $P < 0.05$) between deep sympatry and edge sympatry are indicated by filled symbols (non-significant differences are indicated by open symbols).

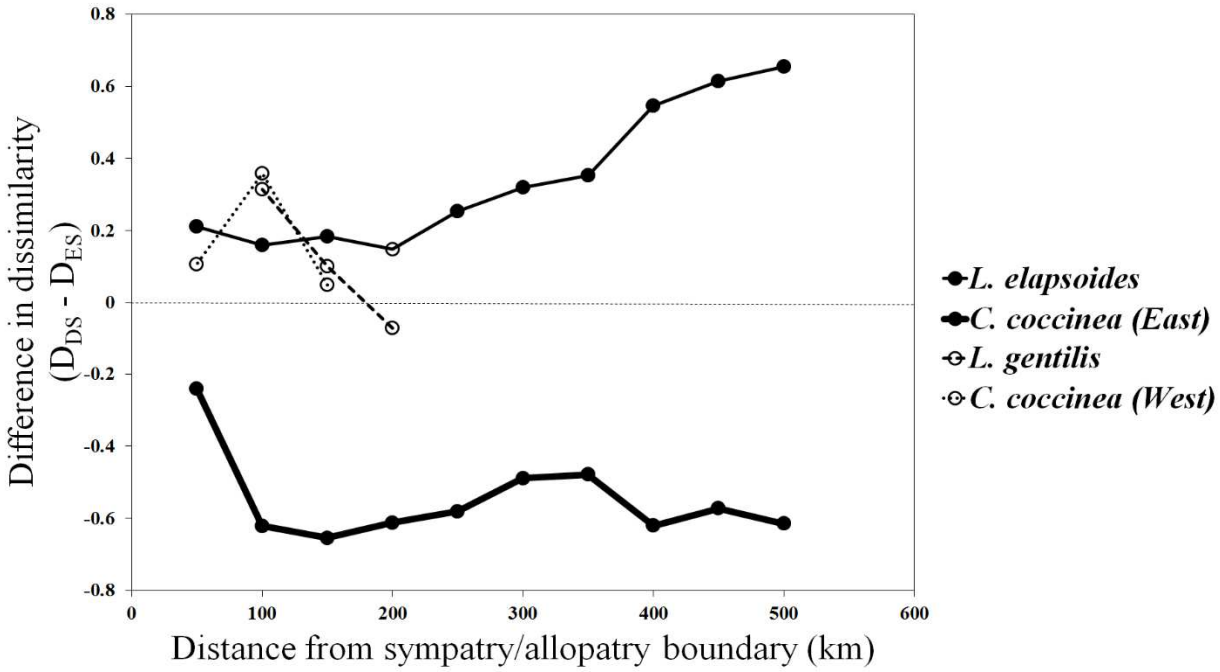


Figure 2.10. Geographic variation in mimetic dissimilarity (D) of (a) *L. elapsoides*, (b) Eastern *C. coccinea*, (c) *L. gentilis*, and (d) Western *C. coccinea* to their models as a function of the distance from the sympatry/allopatry boundary (i.e., 0). More negative values are deeper into sympatry, and more positive values are deeper into allopatry.

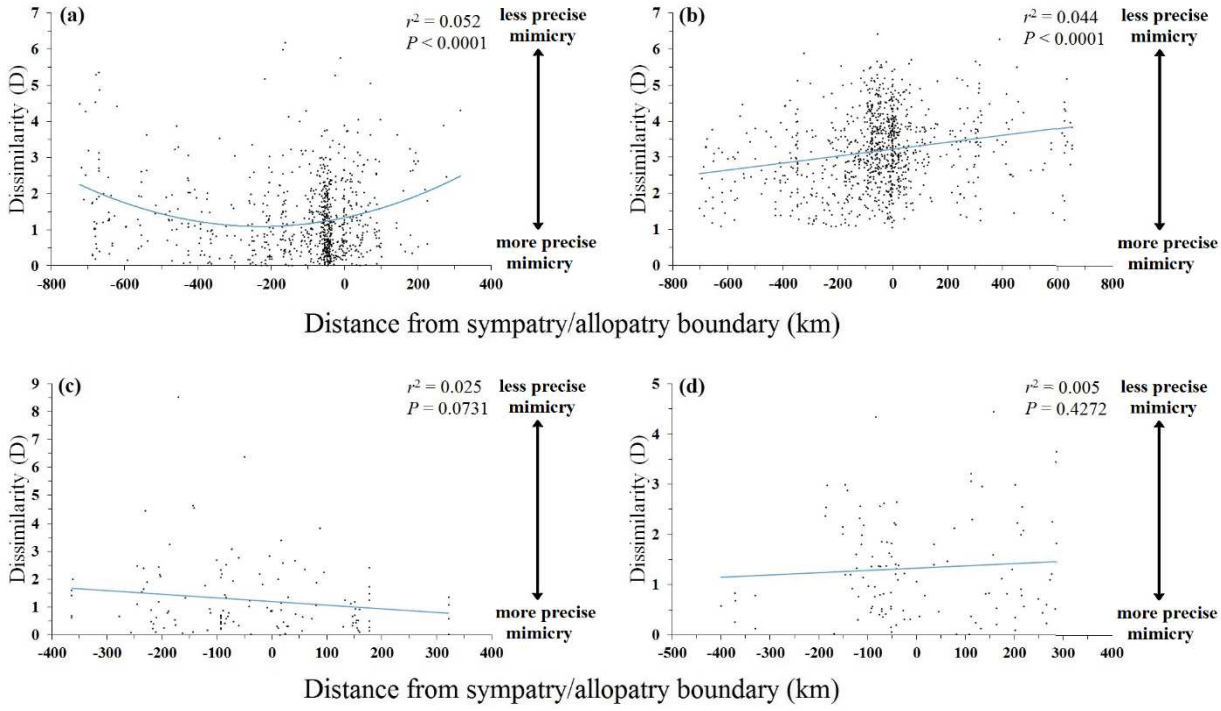


Figure 2.11. Mean dissimilarity of *L. elapsoides*, Eastern *C. coccinea*, *L. gentilis*, and Western *C. coccinea* in sympatry (sym) and allopatry (allo) with their models. Stars indicate means. Box plots show 10th, 25th, 50th (median), 75th, and 90th percentiles. Statistical significance was determined using two-tailed *t*-tests ($P < 0.05$).

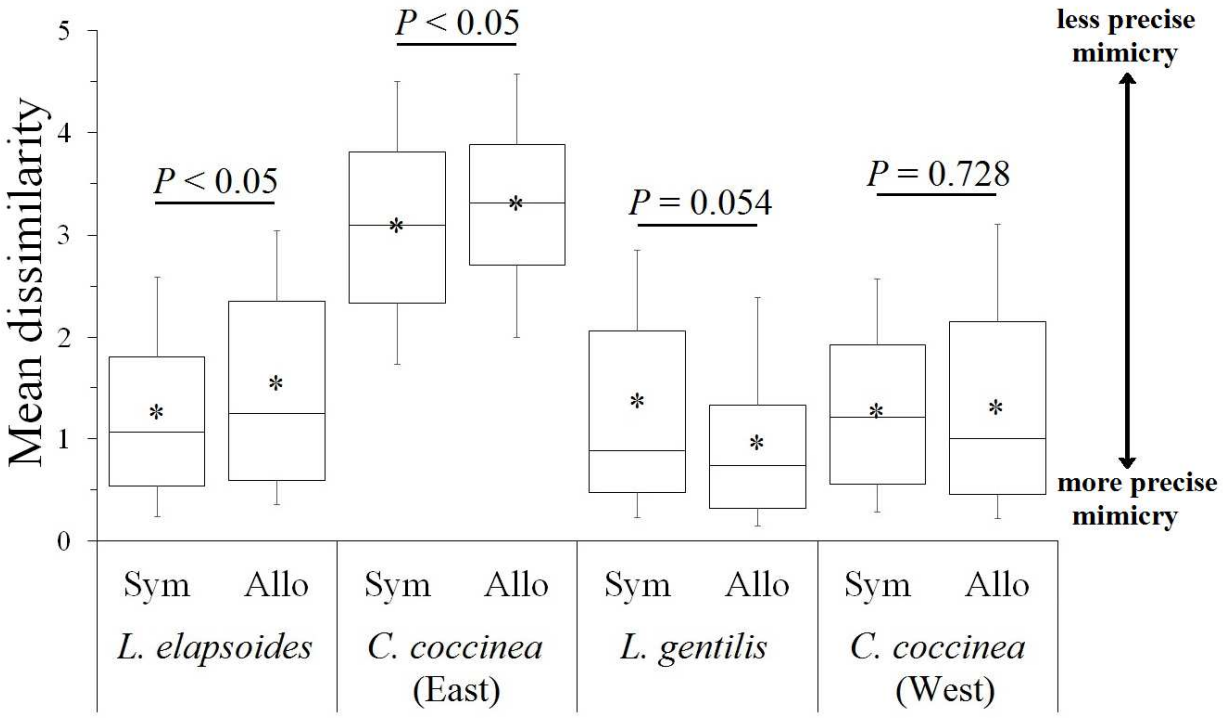


Figure 2.12. Geographic variation in the proportion of dorsum black of (a) L. *elapsoides*, (b) Eastern *C. coccinea*, (c) *L. gentilis*, (d) Western *C. coccinea*, (e) *M. fulvius*, and (f) *M. tener* as a function of latitude.

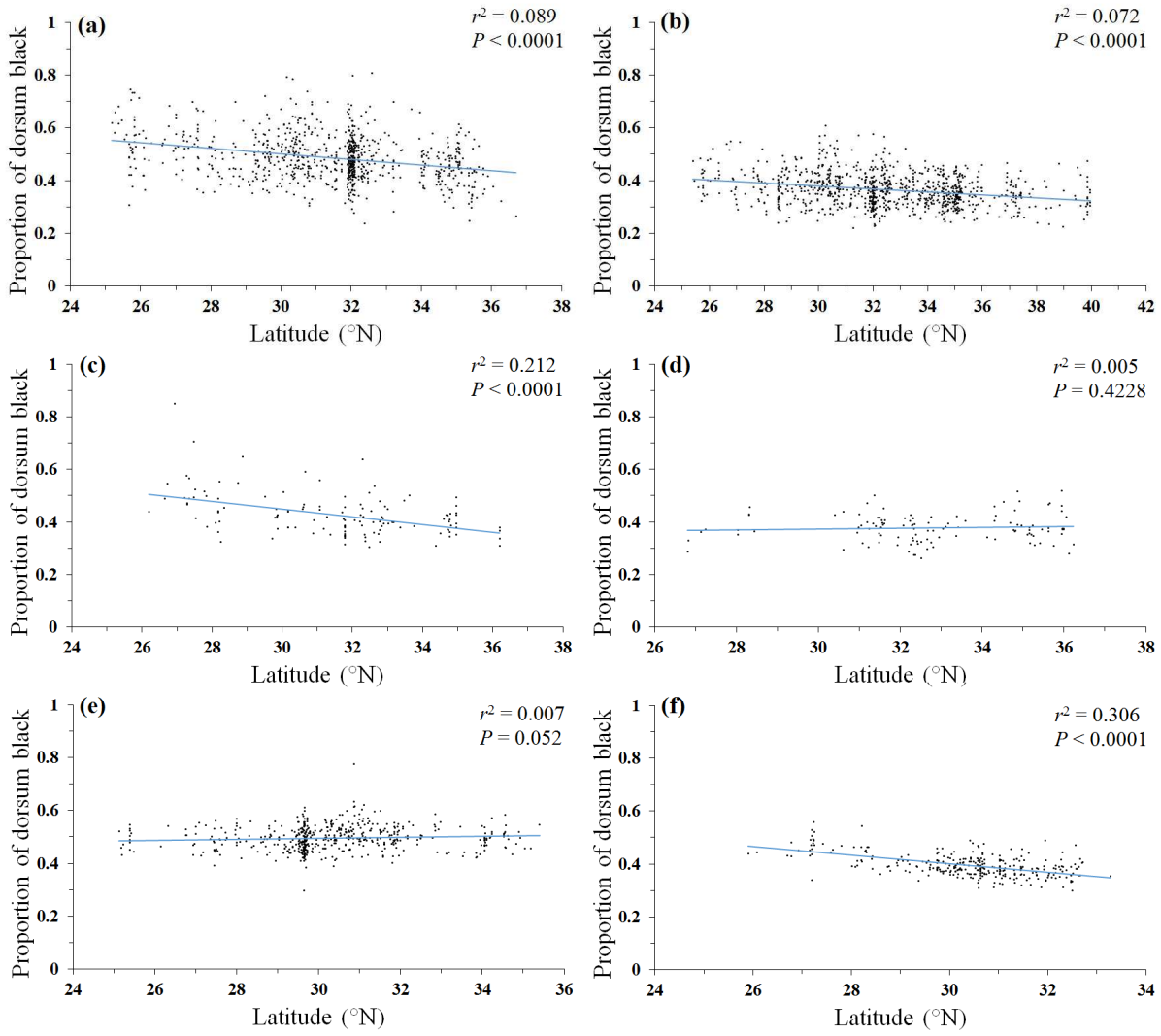


Figure 2.13. Dissimilarity (D) of (a) *L. elapsoides*, (b) Eastern *C. coccinea*, (c) *L. gentilis*, and (d) Western *C. coccinea* as a function of proportion of dorsum black.

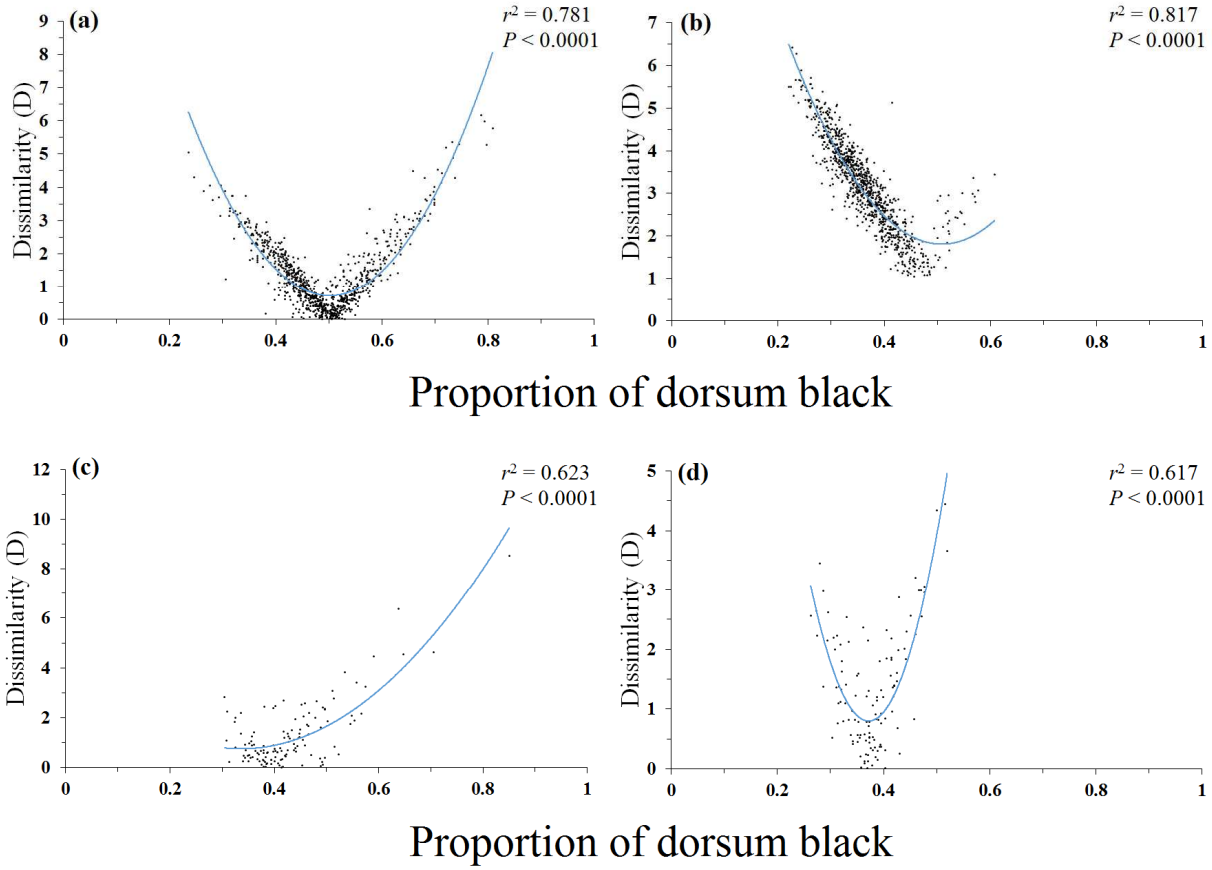
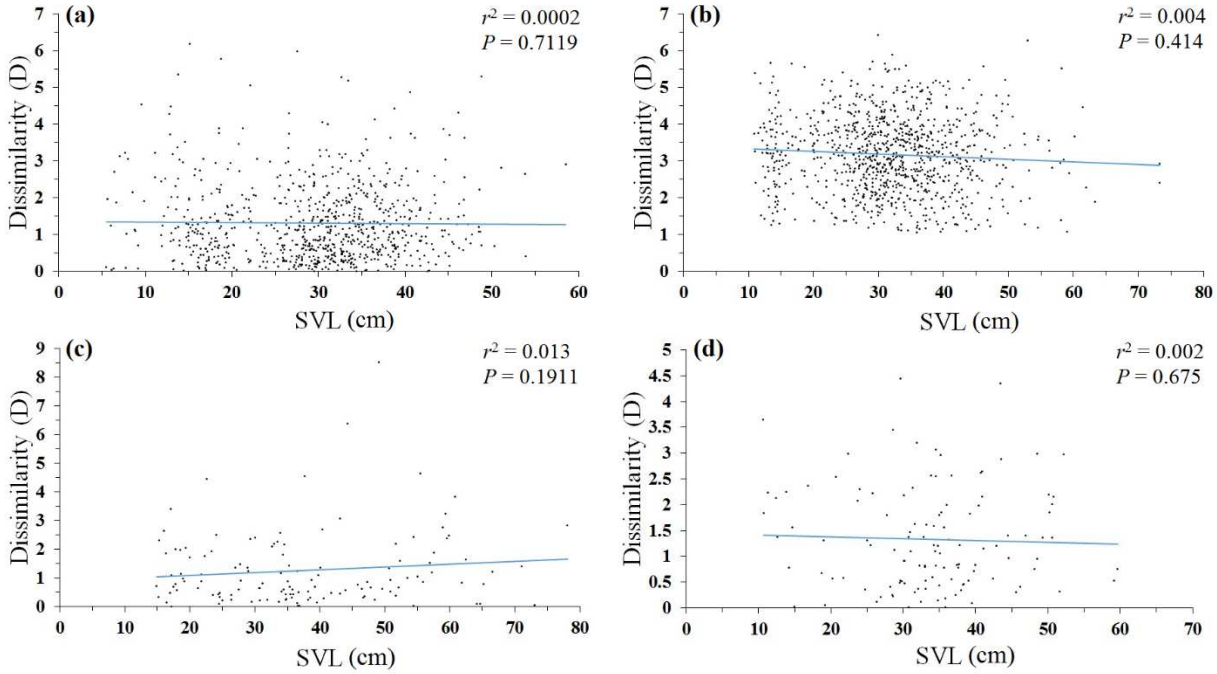


Figure 2.14. Dissimilarity (D) of (a) *L. elapsoides*, (b) Eastern *C. coccinea*, (c) *L. gentilis*, and (d) Western *C. coccinea* as a function of snout-vent length (SVL).



CHAPTER 3: COEVOLUTIONARY ARMS RACES IN BATESIAN MIMICRY? A TEST OF THE CHASE-AWAY HYPOTHESIS[3]

Summary

Reciprocal selection on harmless Batesian mimics and their defended models has long been hypothesized to spawn coevolutionary arms races. Mimics are thought to continually experience selection to better resemble their models. Models are thought to continually experience 'chase-away' selection for phenotypes that let them escape from these 'parasitic' mimics, especially when the cost of having mimics is high. Although abundant evidence suggests that models affect the evolution of mimics, evidence that mimics affect selection on models is meager in natural systems. Here, we tested whether mimics effect selection on models in a snake mimicry complex. Specifically, we tested whether models show phenotypic signatures of chase-away selection in regions that vary in levels of mimetic parasitism, and whether models experience chase-away selection in regions where there are many mimics. Contrary to the predictions of the chase-away hypothesis, we found no evidence that models exhibit phenotypic signatures of chase-away selection or that models experience either directional or disruptive selection favoring extreme phenotypes. The absence of chase-away—in a mimicry complex that appears to be primed for it—suggests that chase-away in natural systems might be weak, rare, or of a different character than has been predicted by theoretical studies of Batesian mimicry.

[3] This chapter previously appeared as an article in the *Biological Journal of the Linnean Society*. The original citation is as follows: Akcali CK, Kikuchi DW, and Pfennig DW. "Coevolutionary arms races in Batesian mimicry? A test of the chase-away hypothesis," *Biological Journal of the Linnean Society* 124, no. 4 (August 2018): 668-676.

Introduction

Coevolutionary arms races—in which a species evolves defenses against its antagonists, spurs these antagonists to invest in counter measures, in turn spurring the focal species to evolve even greater defenses—have long been recognized as important mechanisms of evolutionary change (Ehrlich and Raven 1964; Dawkins and Krebs 1979; Thompson 1994). A relatively understudied context in which coevolutionary arms races have been conjectured to arise is Batesian mimicry (Thompson 1994; Joron and Mallet 1998), wherein harmless prey ('mimics') evolve phenotypic resemblances to defended species that predators avoid ('models'). Because the fitness of such mimics depends on the presence of their models, Batesian mimics are predicted to closely resemble their models phenotypically (Fisher 1930; Ruxton et al. 2004). Likewise, because the efficacy of model warning signals is reduced as the precision and number of mimics increases (this is often referred to as "mimetic load"), models are expected to evolve increasingly distinctive phenotypes that differentiate them from mimics (Poulton 1890; Sherratt and Franks 2005; Franks et al. 2009). This reciprocal interaction between mimics and models might, in turn, lead to a coevolutionary arms race (Van Valen 1973), where mimics continually experience selection to converge on their models to decrease predation, and models continually experience selection to evolve new phenotypes to escape 'parasitic' mimics. Such a process has been dubbed 'chase-away' (Fisher 1930; Gavrilets and Hastings 1998; Joron and Mallet 1998; Franks et al. 2009). Models of chase-away evolution predict that: 1) mimics should experience selection to resemble their models when they are imprecise, and 2) models should experience selection to evolve away from mimics when the mimetic load is high.

There is a marked discrepancy in the empirical support for each of these two predictions of chase-away selection in Batesian mimicry complexes. Although there are many reasons why

mimics might not closely resemble their models (Kikuchi and Pfennig 2013; Sherratt and Peet-Paré 2017), numerous studies have shown that mimics can—and often do—experience selection for precise mimicry (Brodie and Brodie 1980; Brodie 1993; Ohsaki 1995; Pfennig et al. 2001; Caley and Schluter 2003; Harper and Pfennig 2007). In contrast, evidence that models experience chase-away selection is scarce. One study of mimetic avian brood parasites found convincing evidence that models do evolve away from their mimics (although in this case, the signal receiver and the model are one and the same, and mimics are aggressive, not prey; (Spottiswoode and Stevens 2012). An additional study of a salamander-newt mimicry complex found that toxic newts likely experience greater selection for conspicuousness in the presence of their salamander mimics (Kraemer et al. 2015). If conspicuousness is a component of newt warning signals, then this would support chase-away selection. Beyond these studies, there is little evidence of chase-away selection acting on models in Batesian mimicry complexes. This lack of empirical support from natural systems is surprising, given that chase-away dynamics are predicted by many theoretical models of mimicry (Oaten et al. 1975; Gavrilets et al. 1998; Holmgren and Enquist 1999; Franks and Noble 2004; Franks et al. 2009). Furthermore, laboratory experiments (e.g., McGuire et al. 2006; Rowland et al. 2010) have suggested that avian predators may behave in ways that select for chase-away evolution.

We focused on natural populations of a well-characterized Batesian mimicry complex to test for signatures of chase-away. Using morphological analyses, we found that models do not show phenotypic signatures of chase-away selection, and in a field experiment, we found no evidence that models experience chase-away selection. Thus, chase-away might be rarer or of a different character than predicted.

Methods

Study System

The highly venomous, brightly colored eastern coral snake (*Micrurus fulvius*) serves as a model for the nonvenomous scarlet kingsnake (*Lampropeltis elapsoides*) and scarlet snake (*Cemophora coccinea*) (Figure 3.1). Although all three species co-occur in the southeastern United States, both *L. elapsoides* and *C. coccinea* occur well beyond the range of their model (Figure 3.1). Consistent with Batesian mimicry theory, field experiments have shown that mimetic phenotypes of *L. elapsoides* are favored only where *M. fulvius* occurs (i.e., in sympatry); not where *M. fulvius* is absent (i.e., in allopatry) (Pfennig et al. 2001; Pfennig et al. 2007; Harper and Pfennig 2008).

There are at least four reasons to expect chase-away in this mimicry complex. First, the strength of selection on mimics varies geographically. Specifically, selection for precise mimicry is strong on the sympatry/allopatry boundary, as previous field experiments have shown that precise mimetic phenotypes receive more protection from predation than imprecise mimetic phenotypes in this region (Harper and Pfennig 2007; Kikuchi and Pfennig 2010a,b; Pfennig et al. 2015). In contrast, selection for precise mimicry is relaxed in deep sympatry (Harper and Pfennig 2007; Kikuchi and Pfennig 2010a). Second, the ratio of mimics to models varies geographically. On the sympatry/allopatry boundary, mimics greatly outnumber *M. fulvius*; in contrast, the ratio of mimics to *M. fulvius* is low in deep sympatry (Palmer and Braswell 1995; Harper and Pfennig 2007). Third, the rate of evolution of mimic color patterns varies geographically. In at least one population on the sympatry/allopatry boundary, mimics are in the process of rapidly evolving more precise mimicry, whereas mimics in deep sympatry are not evolving at the same rate or in the same direction (Akcali and Pfennig 2014). Fourth, even within a region (e.g., deep

sympatry), mimics vary geographically in mimetic precision, suggesting that different populations might be at different stages of chase-away evolution (Akcali and Pfennig 2017).

Assessing Phenotypic Signatures of Chase-Away Selection

Chase-away selection should be stronger on the sympatry-allopatry boundary where mimetic load is high and where mimics experience strong selection for precise mimicry. In contrast, chase-away selection should weaken further away from this boundary as mimetic load attenuates. Thus, if *M. fulvius* has evolved away from the phenotype of its mimics, then it should geographically vary in phenotype (i.e., populations of *M. fulvius* should be at different stages of chase-away evolution), given that chase-away selection should geographically vary depending on the mimetic load. We quantified the phenotypes of 537 *M. fulvius* throughout their range using previously described methods (Akcali and Pfennig 2014; Akcali and Pfennig 2017). Specifically, we measured the proportions of red and black on the mid-dorsum of each snake. We limited our analysis to red and black because these are the predominant colors on both models and mimics and including yellow would remove the independence of these characters. Previous work has also shown that the proportions of red and black change the most in mimetic snakes (Pfennig et al. 2007; Harper and Pfennig 2008; Akcali and Pfennig 2017), and that these characteristics are targets of predator-mediated selection in one mimetic species (*Lampropeltis elapsoides*) in the southeastern USA (Harper and Pfennig 2007; Kikuchi and Pfennig 2010a,b; Pfennig et al. 2015). For analysis, we combined the proportion of dorsum red and black on models into a common principal component (PC1). We compared the mean and variance of PC1 between different regions using ANOVA and Levene's test, respectively. We tested for differences in PC1 at varying 25-km distances (from 0 to 500+ km) from the sympatry/allopatry

boundary (a 25-km interval was chosen so that the smallest number of snakes in a distance class [15 snakes at 225-250 km] was greater than the number of distance classes). If *M. fulvius* has experienced chase-away evolution, then *M. fulvius* should differ in phenotype between regions where the mimetic load is high (e.g., along the sympatry/allopatry boundary) versus where the mimetic load is low (e.g., deep sympatry).

Assessing Chase-Away Selection

To determine if *M. fulvius* experiences chase-away selection, we measured predation rates on different color pattern phenotypes using artificial snake replicas. This technique has been used successfully to study predation in the field in many taxa and in many regions (Bateman et al. 2017), including the *M. fulvius* mimicry complex (Pfennig et al. 2001; Harper and Pfennig 2007; Pfennig et al. 2007; Kikuchi and Pfennig 2010a,b; Pfennig et al. 2015). Experiments were conducted in southern North Carolina and adjacent South Carolina, USA, where *M. fulvius* reaches its northernmost extent (Powell et al. 2016), and where it experiences its highest known mimetic load (Harper and Pfennig 2007; Kikuchi and Pfennig 2010a). Separate experiments were conducted in the Spring and Fall to evaluate temporal consistency of selection (*M. fulvius* is most active in Spring and Fall; Jackson and Franz 1981).

All replicas were constructed with pre-colored, non-toxic polymer clay (Sculpey III) following previously described protocols (Kikuchi and Pfennig 2010a,b; Pfennig et al. 2015). Replicas bearing three phenotypes of *M. fulvius* were constructed, each of which varied in the proportion of red and black (recall from above that proportions of red and black are targets of predator-mediated selection in this region): 1) a phenotype with average amounts of red and black on *M. fulvius* from the Carolinas ('mean phenotype'), 2) a phenotype with 25% more red

and 25% less black than the average Carolina *M. fulvius* ('red phenotype'), and 3) a phenotype with 25% more black and 25% less red than the average Carolina *M. fulvius* ('black phenotype'). The red and black phenotypes are found in natural populations of *M. fulvius* (Akcali and Pfennig 2017). Thus, these two phenotypes represent extreme, but possible, phenotypes found in natural populations of *M. fulvius*. We constructed 200 replicas per phenotype per season (1200 replicas total).

We hypothesized *a priori* that finding any one of the following three outcomes would suggest that chase-away selection is acting on the color pattern of *M. fulvius*: 1) evidence of *directional* selection favoring the red phenotype (i.e., the red phenotype receives more protection from predation than both the mean and black phenotypes); 2) evidence of *directional* selection favoring the black phenotype (i.e., the black phenotype receives more protection from predation than both the mean and red phenotypes); or 3) evidence of *disruptive* selection favoring both the red phenotype *and* the black phenotype (i.e., the red and black phenotypes both receive more protection from predation than the mean phenotype). Outcomes that would *not* be consistent with chase-away selection would be: 4) evidence of *stabilizing* selection favoring the mean Carolina *M. fulvius* phenotype (i.e., the mean phenotype receive more protection from predation than the red and black phenotypes); or 5) evidence of no selection (i.e., all phenotypes were equally protected from predation).

Each of the three phenotypes were arranged into triads in the field 2 m apart (Pfennig et al. 2001; Harper and Pfennig 2007; Pfennig et al. 2007; Kikuchi and Pfennig 2010a,b; Pfennig et al. 2015). We placed 10 such triads along a transect at each of 20 sites per season. Triads were separated within transects by approximately 50-75 m. After 28-30 days, replicas were collected and scored as having been attacked or not (and whether by a mammal or bird) based on the

presence/absence of tooth and beak marks (Pfennig et al. 2001; Harper and Pfennig 2007; Pfennig et al. 2007; Kikuchi and Pfennig 2010a,b; Pfennig et al. 2015). Previous studies have shown that mammals exhibit avoidance responses to coral snake color patterns (Gehlbach 1972) and that mammalian predators (i.e., black bear, *Ursus americanus*; common raccoons, *Procyon lotor*; gray foxes, *Urocyon cinereoargenteus*; and Virginia opossums, *Didelphis virginiana*) preferentially avoid coral snakes and their mimics, compared to other snakes (Pfennig et al. 2001; Harper and Pfennig 2007; Pfennig et al. 2007; Kikuchi and Pfennig 2010a,b; Pfennig et al. 2015; Akcali et al., unpublished data).

Attack data were analyzed with generalized linear mixed models to compare the fitness of the mean Carolina *M. fulvius* phenotype to that of the other two phenotypes across seasons. Specifically, we used a logistic regression with predation on each replica coded as a binary response variable, the interaction of season and phenotype (both factors) as fixed effects, and the triad within each transect as random effects. The random effects accounts for spatial autocorrelation in predation. We used the likelihood ratio test to verify the significance of fixed effects (Bolker 2008). In the likelihood ratio test (LRT), the predictor of interest is compared with a model that does not include it; a significant difference in model fit between the two is interpreted as support for the inclusion of the predictor. We used chi-squared tests to analyze the relative rates of predation by birds and mammals between Spring and Fall and the absolute difference in total predation pressure between seasons. Analyses were conducted in R (R Core Team 2016).

Results

Assessing Phenotypic Signatures of Chase-Away Selection

Micrurus fulvius varied phenotypically in different regions (ANOVA: $F_{13,536} = 2.533$, $p = 0.0022$; Tukey-Kramer HSD: $p < 0.05$; Figure 3.2); however, phenotype did not depend on distance from the sympatry/allopatry boundary. Individuals from deepest sympatry (where mimetic load is low) did not differ significantly in PC1 from individuals from the sympatry/allopatry boundary (where mimetic load is high; Figure 3.2). Moreover, variation in phenotype did not vary with distance from the sympatry/allopatry boundary (Levene's test, $F = 0.7242$, $p = 0.7398$).

Assessing Chase-Away Selection

Although there was a non-significant trend towards stabilizing selection in the Spring (fewer attacks on the mean phenotype), the three phenotypes were equally likely to be attacked; all three phenotypes were also equally likely to be attacked in the Fall (LRT; $\chi^2_{df=4} = 7.32$, $p = 0.12$; Figure 3.3). Interestingly, there were more replicas attacked in the Fall than the Spring (LRT; $\chi^2_{df=1} = 7.87$, $p = 0.005$; Figure 3.3), with attacks by both avian and mammalian predators higher in the Fall ($\chi^2_{df=1} = 28.46$, $p < 10^{-4}$; Figure 3.4). However, the relative proportion of avian to mammalian predation did not differ between seasons ($\chi^2_{df=1} = 2.53$ $p = 0.11$).

Discussion

We evaluated whether a Batesian mimic and its model participate in a coevolutionary arms race. Batesian mimics and their models have long been hypothesized to be linked in such interactions. Because the efficacy of model warning signals is reduced as the accuracy and the

number of mimics increases, models are expected to evolve increasingly distinctive phenotypes that differentiate them from these 'parasitic' mimics. Thus, if chase-away evolution occurs, then models should differ in phenotype between regions where the mimetic load is high (e.g., along the sympatry/allopatry boundary) versus where it is low (e.g., deep sympatry). Moreover, if chase-away selection occurs, then models should experience selection to escape from mimics when the mimetic load is high (e.g., when mimics are relatively abundant and under selection for more precise mimicry). Our data are inconsistent with both predictions and do not support the hypothesis that Batesian mimics negatively impact the fitness of their models.

Previous work has shown that both *Lampropeltis elapsoides* and *Cemophora coccinea* (Batesian mimics of *Micrurus fulvius*; Fig. 1) vary geographically in mimetic precision depending on the local abundance of *M. fulvius* (Harper and Pfennig 2007; Akcali and Pfennig 2017). Furthermore, previous work has shown that selection for mimicry varies geographically: selection favoring precise mimicry is strongest at the sympatry/allopatry boundary (Figure 3.1; Pfennig et al. 2001; Harper and Pfennig 2007; Kikuchi and Pfennig 2010a,b; Pfennig et al. 2015). These findings therefore confirm that the mimetic load does indeed influence the evolution of mimics. However, in the present study, we did not find the converse: mimetic load did not influence the evolution of models. In particular, we found that *M. fulvius* did vary geographically (Figure 3.2), but *M. fulvius* from regions with high and low mimetic loads did not differ, suggesting that chase-away evolution has not occurred separately in different regions in response to variation in mimetic load. Moreover, we found that the three phenotypes used in our experiments received similar protection from predation in both the Spring and the Fall (Figure 3.3). If this pattern of selection is consistent across years, then the Batesian mimics in our system (*L. elapsoides* and *C. coccinea*) likely have a negligible influence on the evolution of the color

pattern of their model (*M. fulvius*). Thus, our data suggest that mimics and models do not coevolve in this mimicry complex, and that chase-away is unlikely to explain the pronounced geographic variation in mimic-model similarity documented by Akcali and Pfennig (2017).

Predator generalization is theoretically central to driving chase-away, where models that resemble mimics most in phenotype face higher risks of predation (Holmgren and Enquist 1999; Franks et al. 2009). Therefore, one possible explanation for our finding that attack rates did not significantly differ among phenotypes is that their pattern differences were too small to be detected by predators. In other words, predators could have generalized across *all* replicas. However, previous field studies have demonstrated that predators from these same sites respond to even *smaller* differences in *mimic* phenotypes (Kikuchi and Pfennig 2010a,b; Pfennig et al. 2015). Alternatively, predators might have detected differences, but the extreme aposematic phenotypes that we selected were not distinctive enough from the mean phenotype to provide any increased protection from the mimetic load; i.e., our extreme phenotypes might not have represented a coevolutionary 'escape' from mimics. We also consider this possibility unlikely given that a previous field study (Pfennig et al. 2001) conducted at nearby sites suggests that predators do not avoid an even more distinctive phenotype (red, black, and yellow *striped* [rather than banded] pattern) that does not normally occur on any snakes from the southeastern United States. Although it is conceivable that regions of signal space exist that would allow *M. fulvius* to escape from mimics, these peaks in signal space—if they do exist—are likely separated by valleys of low fitness and thus unlikely to be easily evolvable.

The question remains as to whether chase-away selection plays an important role in shaping the color patterns of coral snakes and their mimics generally. The pattern of selection that we documented on *M. fulvius* might be atypical: *M. fulvius* is the most northerly distributed

coral snake and has only two mimic species. Most coral snakes occur in the Neotropics with many more species of mimics (Davis Rabosky et al. 2016b). Chase-away selection might be present in such regions, where model, mimic, and color pattern diversity is much higher. However, our data suggest that chase-away is unlikely to play a widespread role in coral snakes and their mimics. The effect of chase-away on models is predicted to be strongest in regions with a high mimetic load, and—although the mimetic load has not been well-characterized for many diverse tropical assemblages of coral snakes—there might be few locations where coral snakes experience as high of a mimetic load than the region where our study took place. In this region, coral snakes are exceptionally rare, mimics are abundant, and these mimics are relatively precise (Palmer and Braswell 1995; Harper and Pfennig 2007; Akcali and Pfennig 2017). In the Neotropics, by contrast, the difference in the abundance of coral snakes and their mimics is reduced and many of these mimics resemble coral snakes imprecisely (Pough 1988). Thus, the failure to detect chase-away selection in a model that appears to be primed to experience chase-away selection suggests that chase-away is unlikely to contribute to the evolution of color patterns in coral snake and their mimics [note, however, that coral snake species sometimes show substantial geographic variation in color pattern (Greene and McDiarmid 1981), suggesting that mimics might—in at least a few cases—play a role in driving diversification of coral snake color patterns].

More generally, chase-away dynamics might be rare among Batesian mimicry complexes, for at least two reasons. First, mimics should experience stronger selection to converge on their models than models experience to evolve away from their mimics (Nur 1970). Hence, mimics should always evolve faster than their models. This also means that chase-away selection would be most likely to be present if models experience especially strong fitness trade-

offs between phenotypes. For this reason, chase-away might be widespread in egg mimicry complexes involving avian brood parasites and their hosts. In such complexes, hosts face extreme fitness consequences (e.g., the loss of an entire clutch of eggs) if they fail to recognize and reject foreign eggs (Spottiswoode and Stevens 2012). In the context of defensive mimicry, systems in which aversion of aposematic prey is learned by predators should more readily exhibit chase-away dynamics because learning requires sampling, which might increase the strength of directional selection for distinctive model phenotypes (Franks et al. 2009). In contrast, systems in which predators exhibit innate aversion of aposematic prey (as might occur in coral snakes; Smith 1975, 1977) should generally reduce the likelihood that chase-away would occur—especially over short time scales (Franks et al. 2009).

A second reason why chase-away dynamics are likely to be rare in Batesian mimicry complexes is that the warning signals of models (like those of all aposematic species) are generally expected to be under strong *stabilizing* selection (Fisher 1930). Indeed, theory predicts that predation will select for uniformity of warning signals thereby enhancing the ability of predators to recognize and learn to avoid such signals (Ruxton et al. 2004). Consequently, once a warning signal has initially evolved, predator-mediated selection is expected to favor the most common (conspicuous) phenotype (Lindstedt et al. 2011). Such stabilizing selection to maintain the effectiveness of the warning signal might offset any directional or disruptive (chase-away) selection to evolve away from parasitic mimics.

Finally, another critical issue to explore is the significance of temporal variation in predation on the evolution of aposematism. We found that attacks were higher in the Fall than in the Spring (Fig. 4). This might reflect an increased abundance in the Fall of young (potentially, naïve) predators. Indeed, this pattern is consistent with other studies in seasonal environments

(Mappes et al. 2014) that have found that an increase in naïve predators in certain times of the year can make aposematism selectively disadvantageous, as uneducated predators make up a larger proportion of the predator community in the Fall and Winter. This result is also interesting considering that the model, *M. fulvius*, has a slightly higher surface activity peak in Fall than in Spring (Jackson and Franz 1981), whereas the mimics tend to be more surface active during the spring months (Palmer and Braswell 1995). Thus, frequency dependence on the mimic and model patterns might be responsible for driving temporal variation in selection as has been documented in other coral snake mimicry complexes (Cox and Davis Rabosky 2013; Holmes et al. 2017). The ultimate effect of this temporal variation in predation depends on whether the avoidance of coral snake color patterns is innate or learned. Which predators in the southeastern United States exhibit innate or learned avoidance of coral snake color patterns remains to be evaluated.

This study is also consistent with a growing body of evidence that coral snake mimicry often fails to meet general predictions of mimicry theory that frequently apply to other well-characterized mimicry complexes, such as butterflies (Kunte 2009), hoverflies (Penney et al. 2012), and spiders (Caccarelli et al. 2007). Coral snake mimics are more species-rich and abundant than models in much of the New World (Davis Rabosky et al. 2016b). In addition, the genetic architecture of color pattern in coral snake mimics might facilitate the rapid gain and loss of coral snake coloration (Davis Rabosky et al. 2016a). Here, we show that mimics do not appear to affect the evolution of models as would be expected from predictions of chase-away theory.

In sum, although chase-away has long been thought to contribute to the evolution of Batesian mimicry complexes, its general importance in Batesian mimicry—especially, of coral

snakes—remains unclear. More work in natural systems is needed to assess the significance of chase-away mechanisms in the evolution of mimics and models generally.

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Figure 3.1. A coral snake mimicry complex. (*a*) The venomous eastern coral snake (*Micrurus fulvius*) serves as a model for two nonvenomous snake species (*b*), the scarlet kingsnake (*Lampropeltis elapsoides*; top) and the scarlet snake (*Cemophora coccinea*; bottom). (*c*) Both mimics occur well beyond the range of their model in the southeastern United States. Sympatry and allopatry refer to the presence and absence of the model, *M. fulvius*. Range data were downloaded from the IUCN Red List of Threatened Species (www.iucnredlist.org) and were subsequently modified based on point occurrence data obtained from various museums (www.vertnet.org).

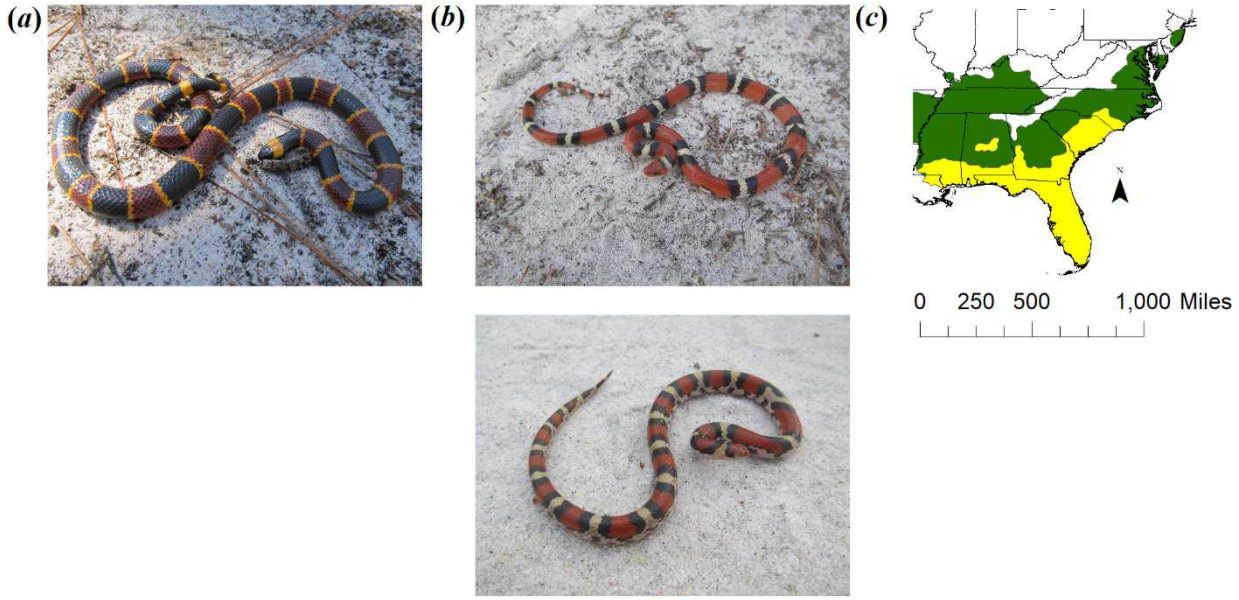


Figure 3.2. Comparison of PC1 (relative proportions of red and black on the dorsum) for *Micrurus fulvius* (the model) at varying distances from the sympatry/allopatry boundary with its mimics. Box plots show 10th, 25th, 50th (median), 75th, and 90th percentiles. Means with different superscripts are significantly different ($p < 0.05$; Tukey-Kramer HSD).

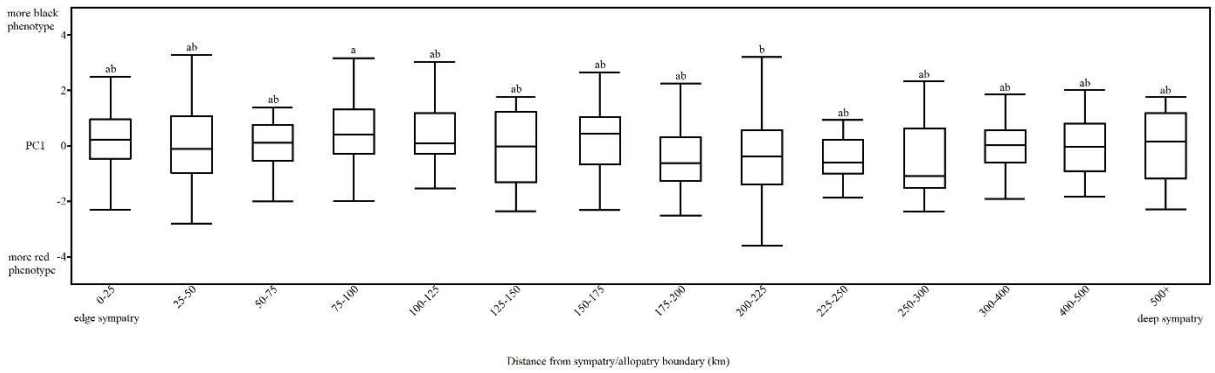


Figure 3.3. Results of field experiments. Shown is the number of attacks on each replica type of Carolina *Micrurus fulvius* in (*a*) Spring 2017, and (*b*) Fall 2017.

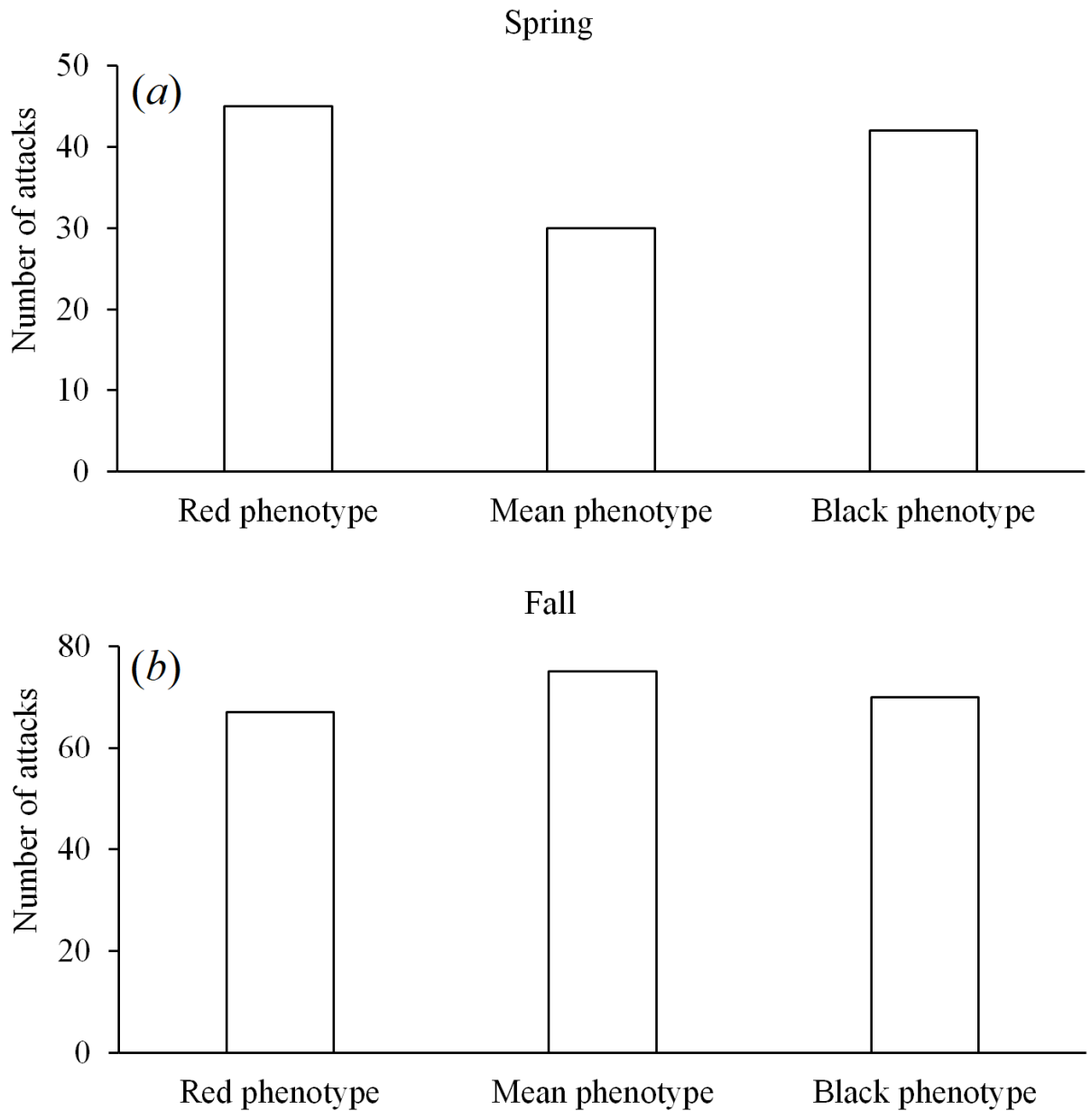
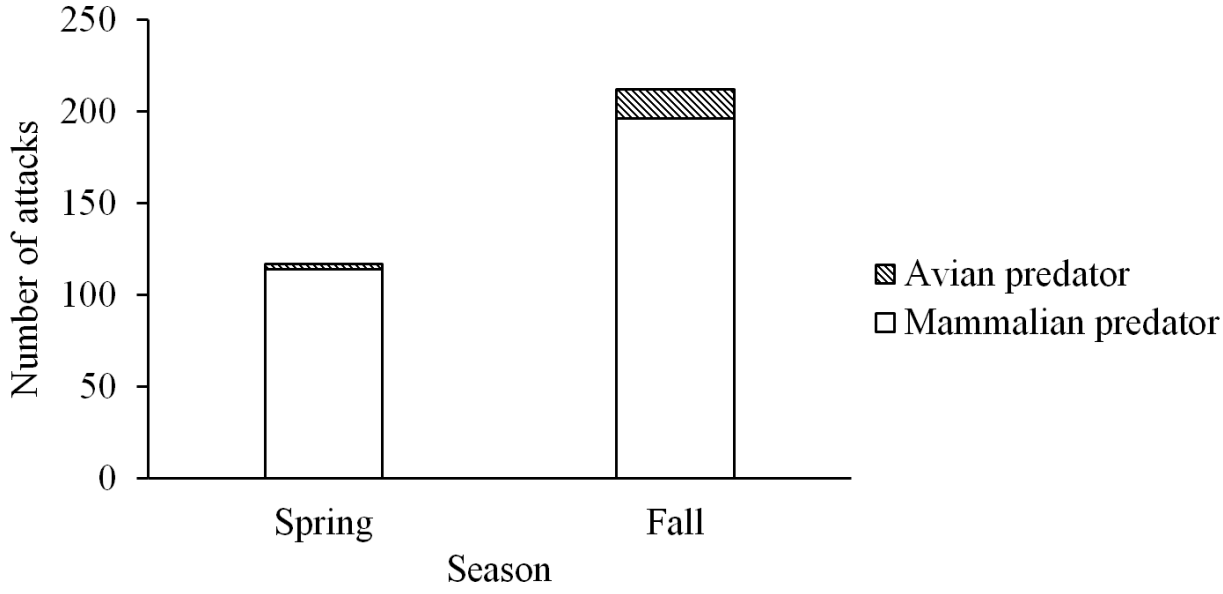


Figure 3.4. Number of avian and mammalian attacks on red, black, and mean phenotypes of North Carolina *Micrurus fulvius* in the Spring and the Fall of 2017.



CHAPTER 4: EVALUATING THE UTILITY OF CAMERA TRAPS IN FIELD STUDIES OF PREDATION[4]

Summary

Artificial prey techniques—wherein synthetic replicas of real organisms are placed in natural habitats—are widely used to study predation in the field. We investigated the extent to which videography could provide additional information to such studies. As a part of studies on aposematism and mimicry of coral snakes (*Micrurus*) and their mimics, observational data from 109 artificial snake prey were collected from video-recording camera traps in three locations in the Americas (*terra firme* forest, Tiputini Biodiversity Station, Ecuador; premontane wet forest, Nahá Reserve, Mexico; longleaf pine forest, Southeastern Coastal Plain, North Carolina, USA). During 1,536 camera days, a total of 268 observations of 20 putative snake predator species were recorded in the vicinity of artificial prey. Predators were observed to detect artificial prey 52 times, but only 21 attacks were recorded. Mammals were the most commonly recorded group of predators near replicas (243) and were responsible for most detections (48) and attacks (20). There was no difference between avian or mammalian predators in their probability of detecting replicas nor in their probability of attacking replicas after detecting them. Bite and beak marks left on clay replicas registered a higher ratio of avian:mammalian attacks than videos registered. Approximately 61.5% of artificial prey monitored with cameras remained undetected by predators throughout the duration of the experiments. Observational data collected from videos

[4] This chapter previously appeared as an article in *PeerJ*. The original citation is as follows: Akcali CK, Pérez-Mendoza HA, Salazar-Valenzuela D, Kikuchi DW, Guayasamin JM, and Pfennig DW. "Evaluating the utility of camera traps in field studies of predation," *PeerJ* 7, (February 2019): e6487.

could provide more robust inferences on the relative fitness of different prey phenotypes, predator behavior, and the relative contribution of different predator species to selection on prey. However, we estimate that the level of predator activity necessary for the benefit of additional information that videos provide to be worth their financial costs is achieved in fewer than 20% of published artificial prey studies. Although we suggest future predation studies employing artificial prey to consider using videography as a tool to inspire new, more focused inquiry, the investment in camera traps is unlikely to be worth the expense for most artificial prey studies until the cost:benefit ratio decreases.

Introduction

Studies of predator-prey interactions are often difficult since natural predation events are challenging to observe (Irschick and Reznick, 2009). Moreover, the ability of the rare observation of single predation events to provide general insights into predator-prey interactions is inherently limited. To overcome both difficulties, artificial replicas of prey species are commonly used to study predation in the wild. Such facsimiles allow key features of prey phenotypes (e.g., color, pattern, shape, or size) to be easily manipulated and produced in large numbers, thereby allowing predation to be studied in diverse natural populations (Irschick and Reznick, 2009). Generally, these studies involve constructing replicas (e.g., of pre-colored, nontoxic clay) bearing different colors, patterns, and shapes and placing several hundred of these replicas in natural habitats, where they are exposed to predation by naturally occurring, free-ranging predators. After a pre-determined period of time, each replica is scored as attacked or not based on the number and type of marks left on it. Conclusions are then drawn based on the patterns of attacks across phenotypes and/or habitats. Such artificial prey techniques have been

used to address a wide variety of evolutionary and ecological questions, ranging from predator psychology to aposematism and mimicry (reviewed in Bateman et al., 2017). These studies have been used to measure predator-mediated natural selection on diverse taxa, including insects (Lövei and Ferrante, 2017), fish (Caley and Schluter, 2003), frogs (Saporito et al., 2007), salamanders (Kuchta, 2005), turtles (Marchand et al., 2002), lizards (Stuart-Fox et al., 2003), snakes (Pfennig et al., 2001), birds (Ibáñez-Alamo et al., 2015), and mice (Vignieri et al., 2010).

This traditional approach of using replicas to study predation in the field has three major shortcomings. First, predation attempts—and the identity of the predators—are inferred (Irschick and Reznick, 2009). Although most marks left by predators permit broad classification of predator type (e.g., beak imprints indicate avian predation), they rarely permit predators to be identified to species (Irschick and Reznick, 2009). Furthermore, replicas can be easily removed by predators, making it impossible to determine if predation even occurred. Second, only a subset of interactions between replicas and predators can be assessed from marks left on replicas (Irschick and Reznick, 2009). For example, predators might detect the replicas and decide not to attack them (Willink et al., 2014). Most studies consider all replicas not bearing attack marks as equivalent in statistical analyses, but a variety of factors can affect the probabilities that predators detect a replica as well as not attack a replica after detecting it. Third, replicas are unlikely to sample all potential predators (Irschick and Reznick, 2009). Predators that rely heavily on movement (e.g., felids) or smell (e.g., canids) to detect prey might ignore motionless or odorless replicas (Irschick and Reznick, 2009). In sum, new and improved insight into predation could be gained from artificial prey studies if additional information on the identity and behavior of predator species could be collected.

Camera trapping technology could provide a potentially useful tool for field studies of

predation. A camera trap consists of a remotely activated camera that is equipped with a motion or an infrared sensor (some also use a light beam as a trigger). This technology has been used in ecological research for decades (Savidge and Seibert, 1988; Griffiths and van Schalk, 1993; O'Connell et al., 2011; Burton et al., 2015), typically to detect or survey the abundance of naturally occurring animals. Although several field studies of predation have experimented with camera trapping techniques, most of these studies have used still images to monitor predator activity (e.g., Picman, 1987; Paluh et al., 2015; Ho et al., 2016; Hanmer et al., 2017) and only a few have used video (Thompson and Burhans, 2004; Latif et al., 2012; Sato et al., 2014; Willink et al., 2014; Jedlikowski et al., 2015; Dziadzio et al., 2016; Figure 4.1). Most these studies using video to monitor predator activity near artificial prey have been conducted on small spatial scales (e.g., at one or a few sites with similar habitat) and have only used videos to aid the identification of predators attacking prey.

Here, we studied the ability of camera trap videos to provide additional information to field studies of predation employing artificial prey. The "prey" in our studies are highly venomous New World coral snakes and various similarly patterned harmless species, which are aposematic and mimetic prey, respectively, bearing conspicuous phenotypes that have long been thought to facilitate the evolution of avoidance behaviors in predators (Bates, 1862; Smith, 1975; Smith, 1977; Figure 4.2). We used camera traps to extract observational data from three independent artificial prey field experiments (Akcali et al., 2018; Supplementary Data). We did so to quantify the frequency at which predators encounter, detect, and attack artificial prey. Using these data, we asked the following questions. First, what are the relative frequencies at which predators encounter, detect, and attack replicas? Second, how do the frequency of encounters, detections, and attacks by predators vary temporally? Third, how does predator type,

avian versus mammal, affect the probability that predators detect and attack artificial prey? Fourth, how does the frequency at which predators encounter, detect, and attack prey vary between predator species? Fifth, how do clay marks and videos differ in their ability to register avian versus mammalian predation attempts? After answering these questions, we conclude by discussing some of the costs and benefits of incorporating videography into field studies of predation.

Methods

Field Experiments

Three field experiments using clay replicas of various species of coral snakes and their presumed mimics (Figure 4.2; Table 4.1) were conducted at three separate locations in the Americas (Figure 4.3). The first experiment was conducted in February 2017 in Amazonian lowland rainforest at Tiputini Biodiversity Station, Orellana, Ecuador (~0°37'S, 76°10'W, 190-270 m asl; Table 4.2). This experiment is a part of a larger study that seeks to understand the causes of diversity in aposematism. In this experiment specifically, the aim was to characterize the pattern of selection on a set of aposematic phenotypes in a region where coral snake diversity is high. The second experiment was conducted from June to July 2017 in premontane wet rainforest at Nahá Reserve, Municipality of Ocosingo, Chiapas, México (~16°58'N, 91°35'W, 800-1200 m asl; Table 4.2). The goal of this experiment was to test the "multiple models hypothesis" of imprecise mimicry, which proposes that mimics might evolve imprecise mimicry as a consequence of a selective trade-off to resemble multiple model species (Edmunds, 2000). The third experiment was conducted from October to November 2017 in longleaf pine forests of the Sandhills and Coastal Plain of North Carolina, USA (~34°45'N, 78°32'W, 0-150 m asl;

Table 4.2). This experiment was a part of a larger study that tested whether a coral snake species and its mimics were engaged in a coevolutionary arms race (Akcali et al., 2018).

Clay replicas in all experiments were constructed using pre-colored, odorless, nontoxic Sculpey III modeling clay. Measurements of preserved snake specimens from several museums (see the specific museum collections listed in Table 4.3) and photographs of live specimens were used to design prey phenotypes in each experiment. Replicas in all experiments were 1-cm in diameter, but varied in length (Table 4.2). Because each field experiment was a part of its own independent study, the experiments varied in several ways (Table 4.2). All damaged replicas were replaced with new replicas during each experiment if transects where checked before their designated date of retrieval (Table 4.2). Sampling effort for each field experiment in terms of replica days was calculated by multiplying the number of days that replicas were left in the field by the total number of replicas that were placed in the field. The latter includes the number of replicas in front of cameras (regardless as to whether the camera was functional or not) as well as the number of replicas without cameras.

Camera Trapping

We used several relatively inexpensive (<USD $100 each) digital camera traps (Spypoint Force 10, Scout Guard SG560V-31B, ANNKE C303, Bestguarder DTC-880V) triggered by an infrared motion-and-heat detector to obtain observational data on predator activity near replicas during each field experiment. Cameras used a variable number of AA batteries and were equipped with 32-gigabyte SD cards. In each experiment, we attached cameras to the trunks of nearby trees and positioned them ~0.75–1 m above the surface of the ground at an approximately 45-degree downward angle. In Ecuador and Mexico, cameras were placed randomly among

transects, approximately one meter away from single replicas and were set to have a high sensitivity (if sensitivity could be altered). In North Carolina, cameras were placed approximately 2 to 3 m in front of sets of three replicas in a clustered fashion (i.e., cameras were placed at every set of replicas in two transects and part of a third transect) and were set to have a medium sensitivity. Average distances between cameras were 1.25 km ± 0.817 km, 1.37 km ± 0.829 km, and 4.60 km ± 4.11 km in Ecuador, Mexico, and North Carolina, respectively. Although vegetation that, when blown by wind, might falsely trigger the cameras was cleared prior to arming the cameras, we tended to place cameras at sites that were devoid of such vegetation to minimize disturbance to the habitat. Cameras were programmed to take 60-second videos when triggered. Videos were associated with data on the location (from GPS), identity of the camera, date, and time. All data collected from camera traps were recorded using data standards developed for the use of camera traps in biodiversity research (Forrester et al., 2016).

Sampling effort for each field experiment in terms of camera days was calculated by taking the sum of the total number of days that each camera was functional in the field. In Ecuador, we placed 27 camera traps (13 Spypoint; 10 Scout Guard; 1 ANNKE) in front of replicas for 14 days. Five camera traps (5 Spypoint) were placed in front of replicas for 8 days and then moved in front of replicas in other transects for the final 6 days. Three cameras (3 Scout Guard) failed to take video throughout the duration of the field experiment and one camera (1 Spypoint) took video for 10 days until a spider built a dense web in front of the lens, making it impossible to make out any animal activity on video thereafter. Thus, cameras in Ecuador were armed for a total of 402 camera days ([23 cameras x 14 days] + [1 camera x 10 days] + [5 cameras x 8 days] + [5 cameras x 6 days]). In Mexico, we placed 22 camera traps (21 Spypoint; 1 ANNKE) in front of replicas for 30 days. One camera (1 ANNKE) failed to take video

throughout the duration of the field experiment. Thus, 21 cameras in Mexico were armed for a total of 630 camera days (21 cameras x 30 days). In North Carolina, we placed 23 cameras (21 Spypoint; 1 ANNKE; 1 Bestguarder) in front of replicas for 28 days. Five cameras (4 Spypoint and 1 ANNKE) failed to take video throughout the duration of the field experiment. Thus, 18 cameras in North Carolina were armed for a total of 504 camera days (18 cameras x 28 days). In Ecuador and Mexico, replicas in front of cameras were often exposed to predation longer than replicas that were not monitored by cameras (Table 1).

Analyses

All vertebrate species that triggered the cameras were recorded. Although a variety of vertebrate species have been documented to prey on coral snakes and their mimics, including frogs, toads, snakes, caimans, hawks, falcons, kestrels, shrikes, anis, puffbirds, skunks, and mustelids (Roze, 1996; Campbell and Lamar, 2004), we focus on potential avian and mammalian predators in this study as reptiles and amphibians were rarely detected on cameras and would likely not be selective agents for aposematic coloration. Furthermore, we excluded potential rodents and lagomorph predators from analyses, as has often been done in previous studies (e.g., Brodie 1993; Kikuchi and Pfennig 2010), as well as non-predatory passerines, doves, and tinamou species, as these species would likely not represent significant threats to real snakes (see list of vertebrate species considered as predators in analyses in Table 4.1). Although our choice of which species to consider as predators might be inaccurate, our focus in this study is on the ability of camera traps to provide additional information. So although we refer to all species captured on videos that might be snake predators as "predators" throughout the manuscript out of convenience, we recognize that it would be more appropriate to refer to many of these predator

species as “potential predators.”

We noted whether each video demonstrated an encounter, detection, attack, and avoidance by a predator. Encounters were simply defined as videos that contained a predator. However, we classified videos of predators as belonging to independent encounters if more than 30 minutes had elapsed between consecutive videos of the same species at the same location. We used 30 minutes as a cut-off because visits by herds of peccaries (*Tayassu pecari* and *Peccari tajacu*) were typically the longest of any species at any given site among the three experimental locations, but most visits were less than 30 minutes. Thus, when we use the term “videos,” we are referring to the unit (i.e., the actual number of videos) that cameras have taken. In contrast, when we use the term “encounter,” we are referring to independent records of predator presence that might include several videos. Detections were defined as encounters where a predator clearly detected a replica (i.e., the predator decreased the rapidity of its movement near the replica and directed attention toward the replica either with its eyes or nose). Attacks were defined as detections where a predator bit a replica. Avoidances were defined as detections that did not result in an attack. Obviously, cases of avoidance may have arisen because a predator failed to recognize a detected replica as a snake but made a decision not to attack. Thus, when we use avoid, we do not make the implicit assumption that predators recognize replicas as snakes.

Prior to reviewing camera records, all replicas with and without associated camera traps were scored in the field as attacked or not attacked, based on the presence or absence of tooth and beak marks, or missing (i.e., no trace of the replica was present). At each replica or sets of replicas with cameras, we then tallied the number of encounters, detections, and attacks by predator species using camera trap videos. We classified predator activity and behavior by hour, starting at midnight, to examine diurnal patterns. Diurnal activity and behavioral patterns were

sufficiently well marked that statistical tests were not needed. We also asked how likely predators were to detect a replica they had encountered, and to attack a replica they had detected. We modeled the probability that a predator would detect a replica given that it had encountered it – i.e., P(Detection|Encounter) and the probability that a detection would result in an attack – i.e., P(Attack|Detection). To obtain a sample size sufficient for analysis, we pooled data across Ecuador and Mexico to analyze P(Detection|Encounter), and across Ecuador, Mexico, and North Carolina to analyze P(Attack|Detection). We used different datasets for these two analyses because in North Carolina, cameras were directed at triads of replicas rather than individual replicas, making the calculation of P(Detection|Encounter) different from that in Ecuador and Mexico. We used the glmer function in the lme4 package to fit logistic regressions of whether or not each encountered replica was detected (or attacked, in the second model) as a function of whether the predator was a bird or a mammal, with transect and replica identity included as random effects. Analyses at the species level were not possible due to the low sample sizes of individual species.

We also asked whether there was a difference in detecting attacks by birds versus mammals using marks left in clay or videos. We tested whether the proportion of attacks by birds versus mammals differed between clay marks and videos using Fisher's Exact Test.

Results

Predator Activity Patterns

After eliminating videos with no identifiable animal or only with people, we had 1,071 videos. After classifying videos not separated by at least 30 minutes per species at a given site as representing single records, we had 906 encounters. After eliminating encounters by species that

were not classified as snake predators, we were left with 268 encounters of 20 predator species (Table 4.4), which included 25 encounters of 6 avian predator species (6 families; Table 4.4) and 243 encounters of 14 mammalian predator species (8 families; Table 4.4).

Across all experimental locations, we found no difference between avian or mammalian predators in their probability of detecting replicas after encounter in Ecuador and Mexico (Figure 4.4; Likelihood ratio test; χ_1^2= 0.2; p = 0.79). We found no difference between avian or mammalian predators in their probability of attacking replicas after detection in Ecuador, Mexico, and North Carolina (Figure 4; Likelihood ratio test; χ_1^2= 0.01; p = 0.92). In total, videos captured 21 attacks and 31 avoidances (Table 4.5).

The frequency of encounters increased approximately 5 and 12 times more rapidly than the frequency of detections and attacks, respectively, as a function of camera trapping effort (Figure 4.5). The frequency of detections increased approximately 2.4 times more rapidly than the frequency of attacks (Figure 4.5).

The timing of encounters, detections, and attacks varied among experimental locations (Figure 4.6). In Ecuador, activity peaked during daylight hours (Figure 4.6). In contrast, in North Carolina, activity peaked at night, with most attacks occurring just after sunset (Figure 4.6). In Mexico, predator encounters were more common at night; however, most detections and attacks occurred during the day (Figure 4.6).

Variation among predator species

The frequency and timing of encounters, detections, and attacks also varied among predator species. In Ecuador, activity was dominated by collared peccaries (*Pecari tajacu*), white-lipped peccaries (*Tayassu pecari*), and gray-winged trumpeters (*Psophia crepitans*)

(80.5% of encounters, 88.9% of detections, and 100% of attacks; Table 4.4). In Mexico, activity was dominated by common opossums (*Didelphis marsupialis*), gray foxes (*Urocyon cinereoargenteus*), and nine-banded armadillos (*Dasypus novemcinctus*) (72.2% of encounters, 100% of detections and attacks; Table 4.4). In North Carolina, activity was mostly restricted to black bears (*Ursus americanus*), common racoons (*Procyon lotor*), Virginia opossums (*Didelphis virginiana*), and gray foxes (97.3% of encounters, 100% of detections and attacks; Table 4.4).

Eleven of 20 predator species (five bird species and six mammal species) that were encountered never detected replicas (Table 4.4). Each of these species was encountered 10 times or less (mean ± s.d.: 2.27 ± 2.72; median = 2; Table 4.4). In contrast, nearly all of the nine species of predator (one bird species and eight mammal species) that detected replicas were commonly encountered near replicas (mean ± s.d.: 26.11 ± 22.40; median = 19; Table 4.4). Species with the highest detection per encounter rates were *Pecari tajacu* (42.3%), *Ursus americanus* (36.8%), and *Urocyon cinereoargenteus* (29.7%) (Table 2). Species with the lowest detection per encounter rates included ocelots (*Leopardus pardalis*; 0.0%), *Didelphis marsupialis* (5.2%), and *Dasypus novemcinctus* (11.1%) (Table 4.4). Of species that detected replicas at least five times, the highest attack per detection rates were by *Urocyon cinereoargenteus* (72.3%) and *Ursus americanus* (71.4%) (Table 4.4). Species with the lowest attack per detection rates were *Pecari tajacu* (0.0%) and *Procyon lotor* (23.5%) (Table 4.4).

Clay marks vs. videos

Using marks left in clay replicas, we observed 33 avian attacks and 21 mammal attacks in Ecuador, 78 avian attacks and 92 mammal attacks in Mexico, and 16 avian attacks and 198

mammal attacks in North Carolina (Figure 4.7). A total of 18, 57, and 12 replicas from Ecuador, Mexico, and North Carolina, respectively, were scored as missing, as we were not able to locate any trace of these replicas at their original location (Figure 4.7). Using video, we observed one avian and one mammal attack in Ecuador, seven mammal attacks in Mexico, and 12 mammal attacks in North Carolina (Figure 4.8; Table 4.5). We found that marks left in clay replicas revealed a significantly higher ratio of avian:mammalian attacks than camera trap videos (Fisher's Exact Test; $p = 0.012$).

Across all experimental locations, 13 replicas that were registered as attacked based on videos were also scored as attacked based on clay marks (Table 4.5). Eight replicas that were registered as attacked based on videos were not scored as attacked using clay marks (Table 4.5). In five of these cases, replicas were scored as missing in the field as videos confirmed that predators removed replicas from their original location. In two cases, replicas were present but no impressions indicative of bite marks were visible. In a final case, one predator attacked a replica without destroying it and another predator later attacked the same replica; thus, this replica was scored as having two attacks according to video but only one attack was scored based on clay marks. No evidence of attacks by predators was obtained from videos for six replicas that were scored as attacked based on clay marks (Table 4.5).

Discussion

We evaluated whether camera trap videos can provide additional information that could be useful to field studies of predation employing artificial prey. Field studies typically rely on the relative frequencies of clay marks on different prey phenotypes to infer avoidance behaviors of predators (e.g., Noonan and Comeault, 2008; Marek et al., 2011; Dell'Aglio et al., 2016;

Kristiansen et al., 2018). Previous predation field studies that have employed camera traps have generally used photography (Figure 1), have been conducted on small scales, and have primarily employed cameras for the sole purpose of identifying predators attacking artificial prey. Our observational data collected from three field experiments conducted in three separate locations show that camera trap videos can be used to provide benefits to field studies of predation beyond predator identification.

Our study demonstrates how data on the frequency at which different predator species encounter, detect, and attack replicas could be gathered using videography. These data could be used in a variety of ways to enhance predation studies employing artificial prey.

First, these observational data could be used to make more robust evaluations of the relative fitness of different prey phenotypes. For example, in heavily shaded habitats such as the tropical forests where field experiments were conducted in Ecuador and Mexico, the warning coloration of coral snakes and their mimics is unlikely to provide protection from predation at night given that the visibility of their color patterns to predators should be low (Kelber et al., 2017). Information on warning coloration is therefore unlikely to factor into decisions by predators to attack replicas at night in such habitats. As a result, an analysis that omitted the two attacks that were observed at night in Mexico (Figure 4.6C) would provide a more robust test of how warning coloration factors into prey-selection decisions by predators. Similarly, because different color pattern phenotypes might vary in their conspicuousness to predators, differences in predation rates could be driven by both variation in prey preference and variation in visual detection rate (Stuart et al., 2012; Rojas et al., 2014). Variation in visual detection rate has been shown to be an unlikely explanation for differences in predation rates between color pattern phenotypes in at least a few aposematic taxa (Brodie, 1993; Wüster et al., 2004; Buasso et al.,

2006; McElroy, 2016). Nevertheless, restricting analyses to replicas that were actually detected would provide more direct tests of the fitness consequences associated with different prey phenotypes, given that the fitness benefits of aposematic prey should only be realized after predators have detected prey. Replicas monitored by cameras across all field experiments more often remain undetected than detected throughout the monitoring period (Table 4.5). Thus, field studies of aposematic prey that limited analyses to the subset of detected replicas could potentially benefit from increased statistical power to resolve differences in predation between phenotypes.

Second, these observational data could be used to more precisely characterize how different predators contribute to selection on prey phenotypes. Although predator communities as a whole did not have a tendency to attack or avoid replicas following detection (Figure 4.4), the data tentatively suggest that predators might vary in their behavioral responses to aposematic phenotypes (Table 4.4). At least one predator species, *P. tajacu*, had a tendency to disproportionately avoid coral snake phenotypes, while most other predator species (e.g., *U. cinereoargenteus*) attacked them (Table 4.4). Given that *P. tajacu* is largely diurnal and is one of the most common predators at Tiputini Biodiversity Station in Ecuador (Blake et al., 2012; Blake and Loiselle, 2018), their contribution to selection might be disproportionately small relative to their abundance. Likewise, *U. cinereoargenteus* is one of the more common mammals encountered during camera trap surveys conducted in the Carolina Sandhills (Akcali et al., unpublished data), where they are largely crepuscular and nocturnal like the coral snake mimics with which they co-occur (Palmer and Braswell, 1995; Whitaker, 1998). Consequently, *U. cinereoargenteus* might have been a key predator in facilitating the recent rapid evolution of a coral snake mimic in the Carolina Sandhills (Akcali and Pfennig, 2014). However, these claims

remain speculative until additional data are gathered that permit a more robust characterization of the prey selection functions of these predators.

Third, observational data from videos could allow more data to be collected from artificial prey experiments. When no traces of a replica can be located at their original location, researchers often conservatively score such replicas as missing and omit them from subsequent analyses (e.g., Kikuchi and Pfennig, 2010; Choteau and Angers, 2011; Lawrence et al., 2018). However, videography—more often than photography—can provide conclusive evidence of cases where missing replicas were due to removal by predators. Across all three experiments, videos revealed that all six replicas that were scored as missing in the field were actually removed by predators. Given that a total of 87 replicas were scored as missing across all three field experiments (Figure 4.7), the potential for videos to rescue lost data might be substantial.

Fourth, these observational data could provide insight into the extent to which artificial prey approaches sample a biased subset of the predator community. Several studies have suggested that avian predators should be more important selective agents on coral snake color patterns than mammalian predators, especially in the tropics (Brodie, 1993; Brodie and Janzen, 1995; Hinman et al., 1997). During our field experiments, avian predators were substantially underrepresented on videos relative to the frequency at which their beak marks were recorded on replicas that were not monitored by cameras (Figure 4.7, Figure 4.8). This pattern is generally consistent with most camera trapping studies that report capture rates for both mammalian and avian species, which have found that avian species tend to have lower capture rates on cameras (e.g., Stein et al., 2008; Blake et al., 2011; Naing et al., 2015). Thus, it is not clear whether this difference in the representation of avian predators in videos and clay marks reflects the fact that avian predators often moved too fast to be recorded on videos, that avian predators detected

replicas outside the field of view of the cameras and actively avoided cameras as a consequence, or alternatively, that this was simply due to the low number of cameras relative to replicas that were not monitored by cameras (Table 4.2). Avian predators and some mammalian predator species (e.g., *L. pardalis*, Table 4.3) might have extremely low rates of detections relative to encounters. Predators with low detection rates might not be capable of being sampled using artificial prey approaches either because replicas do not provide the cues needed for predators to easily detect them or because these predators detect replicas but do not classify them as edible prey. In such cases, laboratory experiments might be necessary to definitely characterize the ability of predators to detect replicas (Rößler et al., 2018). Predator species that are infrequently captured on video would be particularly important for controlled experiments given that low encounter rates ultimately preclude assessment of predator sampling biases of artificial prey.

Thus, videography can provide some additional information for artificial prey studies, but is it worth the costs? An informal survey of predation studies employing artificial prey (see Figure 4.1 for search details) revealed that—out of studies that report both sample sizes and the length of time artificial prey were exposed to natural predators ($N = 441$ studies)—most employ large numbers of replicas (mean ± s.d. = 482 ± 712, median = 300) for an exposure period close to two weeks (mean ± s.d. = 12.7 ± 9 days, median = 12 days). Although the amount of information provided by videos varied substantially among our experiments (Figure 4.8, Table 4.5), one camera, averaged across all three experiments, obtained 0.18 encounters, 0.04 detections, and 0.01 attacks per day by species that we classified as predators. If these frequencies are calculated over a single transect consisting of 30 video-monitored replicas, which would represent 10% of the total replicas employed in the median artificial prey experiment, over a 12-day study timeline, representing the length of the median artificial prey experiment, a

total of 65.3 encounters, 13.7 detections, and 4.8 attacks would be expected to be observed. If each camera were to cost USD $100, each additional encounter, detection, and attack in terms of camera expenses would cost approximately USD $46, $219, and $625, respectively. If these figures were to be calculated for avian predators alone, a total of 7.1 encounters, 1.2 detections, and 0.3 attacks would be expected for a single 30-replica transect monitored by cameras for 12 days, with each additional encounter, detection, and attack requiring USD $423, $2,500, and $10,000, respectively, in camera costs. Thus, obtaining additional information via videography can be relatively expensive even without considering its accompanying logistical and time costs, which are not negligible but relatively minor comparatively speaking (Table 4.6). Indeed, the cost of cameras that was incurred for each of our field experiments was more than the total cost of conducting any one experiment without cameras (Table 4.6). The reliability of video recording can impose additional costs, as six out of 18 replicas monitored by cameras bore clay marks by predators but no evidence of predation was captured on video.

In other systems, however, these costs might not be quite as high. If the percent of replicas attacked per day is used as a proxy for predator activity, the average predator activity level from our three experiments (ca. 1% replicas/day) was lower compared to other artificial prey studies (mean = 6% replicas/day, median = 4% replicas/day, N = 424 studies). If we recalculate the amount of information and costs that would be expected for a single transect of the median artificial prey study (30 camera-monitored replicas for 12 days) assuming that differences in encounters, detections, and attacks are proportional to differences in encounters, detections, and attacks that were estimated in our study, a total of 98 encounters, 20.6 detections, and 7.2 attacks would be expected, with each additional encounter, detection, and attack requiring approximately USD $31, $146, and $416, respectively, in camera costs. If these same

calculations and assumptions are made using each of the predation rates that have been reported from our informal literature survey, the minimum level of predator activity (in terms of % predation per day) necessary for the purchase of one additional camera to capture an additional encounter, detection, or attack would be approximately 0.01%, 0.03%, and 0.08% replicas/day, respectively (Figure 4.9). Approximately 68.3% of artificial prey studies have reported predator activity levels higher than the 0.03% threshold, whereas only 18.2% of such studies have reported predator activity levels higher than the 0.08% threshold. Unless measures are taken to increase the rate at which information could be obtained (e.g., increasing the realism of replicas; Paluh et al. 2014), the benefits of additional information would only be worth the cost of cameras in a minority of systems.

Results from this study provide quantitative estimates of the amount of additional information that camera trap videos could provide to artificial prey studies and demonstrates some of the benefits of using videography over remote photography in artificial prey studies. Across three field experiments, dozens of observations were obtained on the frequency at which predators encounter, detect, attack, and avoid artificial prey. Observations of predator activity were dominated by mammals. Videography likely underestimates activity by avian predators as marks on artificial prey registered a higher ratio of avian:mammalian attacks than videos. These observational data can be used to estimate the rates and probabilities of encounters, detections, attacks, and avoidances by predators. This information could then be used to conduct more direct tests of the relative fitness of different artificial prey phenotypes as well as provide insight into the relative contribution of different predator species to selection on prey. However, the incorporation of cameras into artificial prey studies that experience low rates of predator activity would be difficult to justify given the current costs of cameras. Nevertheless, videography would

still prove useful at smaller scales as a tool to generate new observations that could lead to new questions or ideas for testing.

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Table 4.1. List of study snake species and all vertebrate species detected from camera trap videos at each experimental location. Species in bold were considered to be potential predators of snakes in analyses. Nomenclature follows Ridgely and Greenfield (2001), Wilson and Reeder (2005), Peterson (2010), and Vallely and Dyer (2018).

Ecuador		Common Name	Scientific Name
	Snake		
		South American coral snake	*Micrurus lemniscatus*
		Ornate coral snake	*Micrurus ornatissimus*
		Worm-eating coral snake	*Micrurus ortoni*
		Amazonian coral snake	*Micrurus spixii*
		Aquatic coral snake	*Micrurus surinamensis*
	Bird		
		Brown nunlet	***Nonnula brunnea***
		Slate-colored hawk	***Buteogallus schistaceus***
		Gray-winged trumpeter	***Psophia crepitans***
		Sapphire quail-dove	*Geotrygon saphirina*
		Ruddy quail-dove	*Geotrygon montana*
		Great tinamou	*Tinamus major*
		White-throated tinamou	*Tinamus guttatus*
		Variegated tinamou	*Crypturellus variegatus*
		Undulated tinamou	*Crypturellus undulates*
		Cinereous tinamou	*Crypturellus cinereus*
		Gray-fronted dove	*Leptotila rufaxilla*
		Rufous-capped antthrush	*Formicarius colma*
		White-necked thrush	*Turdus albicollis*
	Mammal		
		Nine-banded armadillo	***Dasypus novemcinctus***
		Giant armadillo	***Priodontes maximus***
		Ocelot	***Leopardus pardalis***
		White-lipped peccary	***Tayassu pacari***
		Collared peccary	***Peccari tajacu***
		South American red brocket deer	*Mazama americana*
		Lowland paca	*Cuniculus paca*
		Black agouti	*Dasyprocta fuliginosa*
		Unidentified small rodents	*Rodentia* spp.
	Reptile		
		Forest whiptail	*Kentropyx pelviceps*
Mexico		Common Name	Scientific Name

	Snake		
		Variable coral snake	*Micrurus diastema*
		Elegant coral snake	*Micrurus elegans*
		Variegated false coral snake	*Pliocercus elapoides*
	Bird		
		Lesson's motmot	***Momotus lessonii***
		Orange-billed sparrow	*Arremon aurantiirostris*
		Spotted wood-quail	*Odontophorus guttatus*
		Slaty-breasted tinamou	*Crypturellus boucardi*
		Great tinamou	*Tinamus major*
		Little tinamou	*Crypturellus soui*
		Ruddy quail-dove	*Geotrygon montana*
		Gray-headed dove	*Leptotila plumbeiceps*
		White-bellied wren	*Uropsila leucogastra*
		Clay-colored thrush	*Turdus grayi*
		Plain chachalaca	*Ortalis vetula*
		Hummingbirds	*Trochilidae* sp.
	Mammal		
		Tayra	***Eira barbara***
		Ocelot	***Leopardus pardalis***
		Jaguarundi	***Puma yagouaroundi***
		Common raccoon	***Procyon lotor***
		White-nosed coati	***Nasua narica***
		Hooded skunk	***Mephitis macroura***
		Gray fox	***Urocyon cinereoargenteus***
		Nine-banded armadillo	***Dasypus novemcinctus***
		Common opossum	***Didelphis marsupialis***
		Lowland paca	*Cuniculus paca*
		Central American agouti	*Dasyprocta punctata*
		Squirrels	*Sciurus* sp.
		Unidentified small rodents	*Rodentia* spp.
	Reptile		
		Blue spiny lizard	*Sceloporus serrifer*
		Jungle runners	*Ameiva* sp.
North Carolina		Common Name	Scientific Name
	Snake		
		Eastern coral snake	*Micrurus fulvius*
	Bird		
		American crow	***Corvus brachyrhynchos***
		Wild turkey	***Meleagris gallopavo***
		Hermit thrush	*Catharus guttatus*
		Northern cardinal	*Cardinalis cardinalis*
	Mammal		
		Gray fox	***Urocyon cinereoargenteus***
		Virginia opossum	***Didelphis virginiana***
		Common raccoon	***Procyon lotor***

	Black bear	***Ursus americanus***
	Fox squirrel	*Sciurus niger*
	Eastern gray squirrel	*Sciurus carolinensis*
	White-tailed deer	*Odocoileus virginianus*
	Eastern cottontail	*Sylvilagus floridanus*

Table 4.2. Field experiments. List of characteristics of field experiments that aimed to test hypotheses of aposematism and mimicry in Ecuador, Mexico, and North Carolina, USA. Camera traps were employed at a subset of replicas to collect observational data on predator activity near artificial prey replicas.

	Ecuador	**Mexico**	**North Carolina, USA**
Number of phenotypes	5 (4 *Micrurus* variants + brown control)	4 (3 *P. elapoides* variants + brown control)	3 (3 *M. fulvius* variants)
Length of replicas	165 mm	250 mm	180 mm
Number of transects	27	35	20
Minimum distance between transects	200 m	200 m	3 km
Placement of replicas in transects	Singly, along forest trails, and 1-4 m off trails on alternating sides	Singly, along forest trails, and 1-4 m off trails on alternating sides	Each variant in groups of three off trails; all replicas attached to nails
Distance between replicas or sets of replicas	5-10 m	5-10 m	50-75 m
Replicas with cameras	37	22	69
Replicas without cameras	1,313	1,378	531
Days replicas without cameras left in field	6	12	28
Days replicas with cameras left in field	6, 8, or 14	30	28
Replica days	8,356	17,196	16,800
Interval replicas were checked	2 days	6 days	Replicas not checked during experiment

Table 4.3. List of museums. Museums that contributed specimens that were used to aid the construction of artificial snake replicas.

Inst. Code	Institution Name
AMNH	American Museum of Natural History, New York City, NY
AUMNH	Auburn University Natural History Museum and Learning Center, Auburn, AL
BRTC	Texas Cooperative Wildlife Collection, now Biodiversity Research and Teaching Collections, Texas A&M University, College Station, TX
BYU	Monte L. Bean Life Science Museum, Brigham Young University, Provo, UT
CM	Carnegie Museum of Natural History, Philadelphia, PA
FLMNH	Florida Museum of Natural History, Gainesville, FL
FMNH	Field Museum of Natural History, Chicago, IL
GSU	Georgia State University, Statesboro, GA
INHS	Illinois Natural History Survey, University of Illinois, Champaign, IL
LSU	Louisiana Museum of Natural History, Louisiana State University, Baton Rouge, LA
MISS	Mississippi Museum of Natural History, Jackson, MS
MPM	Milwaukee Public Museum, Milwaukee, WI
NCSM	North Carolina State Museum of Natural Sciences, now the North Carolina Museum of Natural Sciences, Raleigh, NC
OMNH	Sam Noble Oklahoma Museum, University of Oklahoma, Norman, OK
QCAZ	Zoology Museum, Pontifical Catholic University of Ecuador, Quito, Ecuador
UMNH	Utah Museum of Natural History, University of Utah, Salt Lake City, UT
USNM	Smithsonian National Museum of Natural History, Washington, D.C.
UTA	University of Texas at Arlington Amphibian and Reptile Diversity Research Center, Arlington, TX
UTEP	The Centennial Museum, University of Texas at El Paso, El Paso, TX

Table 4.4. Predator species. Frequency of encounters, detections, and attacks by each snake predator species observed from camera trap videos during three field experiments conducted in Ecuador, Mexico, and North Carolina, USA, that were aimed to test hypotheses of aposematism and mimicry. Nomenclature follows Ridgely and Greenfield (2001), Wilson and Reeder (2005), Peterson (2010), and Vallely and Dyer (2018).

	Ecuador			
Family	**Common Name (Scientific Name)**	**Encounters**	**Detections**	**Attacks**
Bucconidae	Brown nunlet (*Nonnula brunnea*)	2		
Tayassuidae	Collared peccary (*Peccari tajacu*)	26	11	
Dasypodidae	Giant armadillo (*Priodontes maximus*)	1		
Psophiidae	Gray-winged trumpeter (*Psophia crepitans*)	16	4	1
Dasypodidae	Nine-banded armadillo (*Dasypus novemcinctus*)	6	2	
Felidae	Ocelot (*Leopardus pardalis*)	2		
Tayassuidae	Peccary sp.	8		
Accipitridae	Slate-colored hawk (*Buteogallus schistaceus*)	2		
Tayassuidae	White-lipped peccary (*Tayassu pacari*)	4	1	1
	Total	**67**	**18**	**2**
	Mexico			
Family	**Common Name (Scientific Name)**	**Encounters**	**Detections**	**Attacks**
Didelphidae	Common opossum (*Didelphis marsupialis*)	19	1	1
Procyonidae	Common racoon (*Procyon lotor*)	1		
Canidae	Gray fox (*Urocyon cinereoargenteus*)	8	6	6
Mephitidae	Hooded skunk (*Mephitis macroura*)	1		
Felidae	Jaguarundi (*Puma yagouaroundi*)	1		
Momotidae	Lesson's motmot (*Momotus lessonii*)	1		
Dasypodidae	Nine-banded armadillo (*Dasypus novemcinctus*)	12		
Felidae	Ocelot (*Leopardus pardalis*)	8		
Mustelidae	Tayra (*Eira barbara*)	2		
Procyonidae	White-nosed coati (*Nasua narica*)	1		
	Total	**54**	**7**	**7**
	North Carolina, USA			
Family	**Common Name (Scientific Name)**	**Encounters**	**Detections**	**Attacks**
Corvidae	American Crow (*Corvus brachyrhynchos*)	2		
Ursidae	Black bear (*Ursus americanus*)	19	7	5
Procyonidae	Common racoon (*Procyon lotor*)	80	17	4

Canidae	Gray fox (*Urocyon cinereoargenteus*)	29	5	2
Didelphidae	Virginia opossum (*Didelphis virginiana*)	15	3	1
Phasianidae	Wild Turkey (*Meleagris gallopavo*)	2		
	Total	**147**	**32**	**12**

Table 4.5. Camera trap observations. Frequency of encounters, detections, and attacks are in behavior events/100 camera days (total number of observations is given in parentheses). Number of camera days is given below the site headings. Numbers of encounters, detections, and attacks are based on records separated by at least 30 min (for a given species at a given site).

	Ecuador	**Mexico**	**North Carolina**	**Total**
	402	630	504	1,536
Predator encounters	16.7 (67)	8.6 (54)	29.2 (147)	17.4 (268)
Mammalian predator encounters	11.7 (47)	8.4 (53)	28.4 (143)	15.8 (243)
Avian predator encounters	5.0 (20)	0.2 (1)	0.8 (4)	1.6 (25)
Detections	4.0 (16)	1.1 (7)	6.3 (32)	3.4 (52)
Mammalian predator detections	3.0 (12)	1.1 (7)	6.3 (32)	3.1 (48)
Avian predator detections	1.0 (4)			0.3 (4)
Attacks	0.5 (2)	1.1 (7)	2.4 (12)	1.4 (21)
Mammalian attacks	0.2 (1)	1.1 (7)	2.4 (12)	1.3 (20)
Avian attacks	0.2 (1)			0.1 (1)
Attacks recorded on clay but not cameras	0.2 (1)		0.99 (5)	0.39 (6)
Attacks recorded on cameras but not clay	0.5 (2)	0.63 (4)	0.4 (2)	0.52 (8)
Attacks recorded on both cameras and clay		0.48 (3)	1.98 (10)	0.78 (12)
Number of replicas with functional cameras	34	21	54	109
Number of undetected replicas	24	14	29	67
Number of marks on replicas with cameras		3	15	18

Table 4.6. Costs of conducting artificial prey field experiments with and without camera traps. Equipment and supply costs represent the actual costs for listed items. Personnel wages were assumed to be equivalent for all experiments. For Ecuador and Mexico, travel and lodging costs represent the actual costs that were expended to travel to and from field sites. For North Carolina, a standard government travel rate was used to estimate travel costs.

Ecuador	**Without Cameras**	**Cost ($)**	**Added Cost of Cameras**	**Cost ($)**
Equipment and Supplies	20 kg Sculpey III Clay	478	21 Spypoint Force 10	1,890
	1350 pieces of 20 gauge 18'' stem wire	95	10 Scout Guard SG560V-31B	900
	Polymer clay extruder	110	1 ANNKE C303	80
	Pliers	5	32 SD Cards (32 GB)	320
			214 AA batteries	64
Personnel ($15/hour)	Replicas checked every 2 days	840	Extra baggage fee	150
			Data processing (9 hours)	135
Travel	Airfares	1,124	Total	**$3,539**
	Ground Transportation	120		
Lodging	14 nights	644		
	Total	**$3,416**	**Total for Experiment**	**$6,955**
Mexico	**Without Cameras**	**Cost ($)**	**Added Cost of Cameras**	**Cost ($)**
Equipment and Supplies	24 kg Sculpey III Clay	575	21 Spypoint Force 10	1,890
	1400 pieces of 20 gauge 18'' stem wire	98	1 ANNKE C303	80
	Polymer clay extruder	110	22 SD Cards (32 GB)	220
	Pliers	5	134 AA batteries	42
Personnel ($15/hour)	Replicas checked every 6 days	600	Extra baggage fee	150
			Data processing (12 hours)	180
Travel	Airfare	555	Total	**$2,562**
	Ground Transportation	250		
Lodging	30 days	100		
	Total	**$2,293**	**Total for Experiment**	**$4,855**
North Carolina, USA	**Without Cameras**	**Cost ($)**	**Added Cost of Cameras**	**Cost ($)**
Equipment and Supplies	12 kg Sculpey III Clay	287	21 Spypoint Force 10	1,890

	600 pieces of 20 gauge 18'' stem wire	42	1 ANNKE C303	80
	Polymer clay extruder	110	1 Bestguarder DTC-880V	90
	Pliers	5	23 SD Cards (32 GB)	230
	200 6-in 2 gauge nails	80	142 AA batteries	44
	200-g fishing line	15	Data processing (11 hours)	165
Personnel ($15/hour)	Replicas picked up after 28 days	240		
			Total	**$2,499**
Travel ($0.54/mile)	Two 400-mile trips	432		
	Total	**$1,211**	**Total for Experiment**	**$3,710**

Figure 4.1. Field studies of predation. Number of field studies of predation employing camera traps using different types of monitoring methods and different types of artificial prey. Manuscripts were informally searched in Google Scholar (http://scholar.google.com) using a variety of search terms (e.g., artificial prey, artificial nest, clay model, and predation) and taxon terms (e.g., amphibian, bird, butterfly, frog, lizard, salamander, and snake). The search was conducted 23 December 2017.

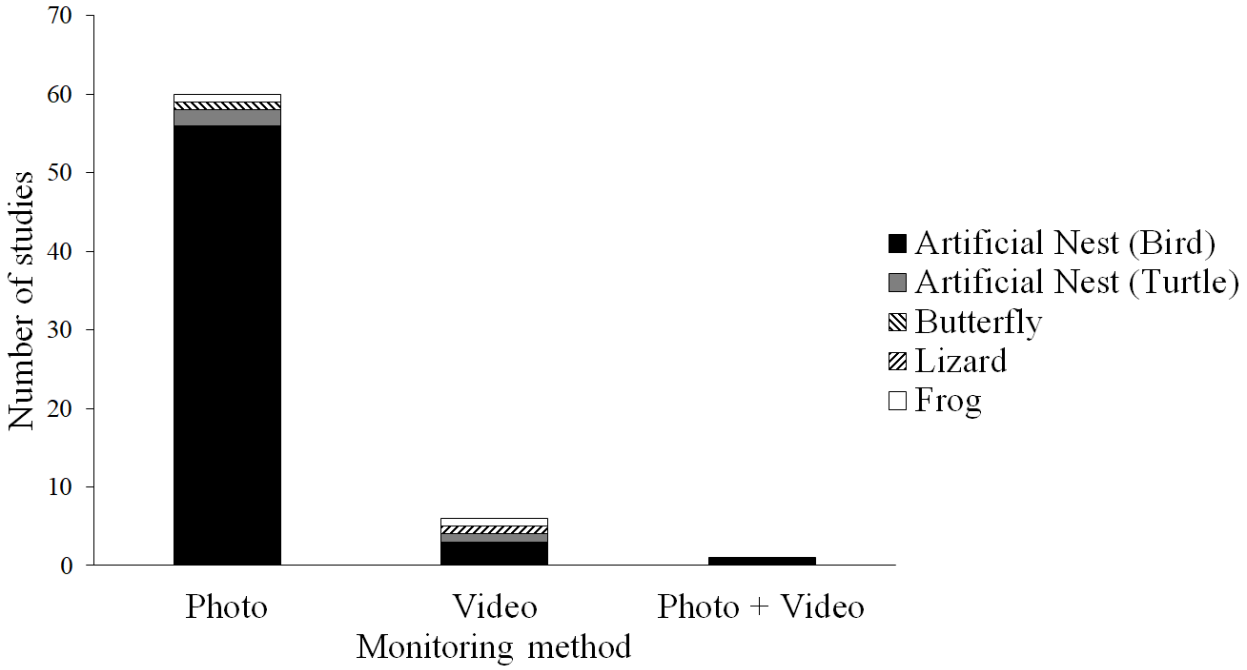

Figure 4.2. Study snake species. A sampling of images of live snakes (A, C, E) and artificial snake replicas (B, D, F) from each experimental location. (A, B) The South American coral snake (*Micrurus lemniscatus*) (photo credit: Mike Pingleton), (C, D) the variable coral snake (*Micrurus diastema*) (photo credit: Eric Centenero Alcalá), and (E, F) the eastern coral snake (*Micrurus fulvius*) (photo credit: Christopher K. Akcali). Note the bite marks and change in shape caused by a mammalian predation attempt in D.

Figure 4.3. Study areas. Camera traps were used to collect observational data on predator behavior in three field experiments, conducted in North Carolina, USA, Mexico, and Ecuador, that were aimed to test hypotheses of aposematism and mimicry. Insets show habitat typical of the study areas: (A) longleaf pine forest, North Carolina, USA; (B) premontane wet forest, Chiapas, Mexico; and (C) *terra firme* rainforest, Orellana, Ecuador.

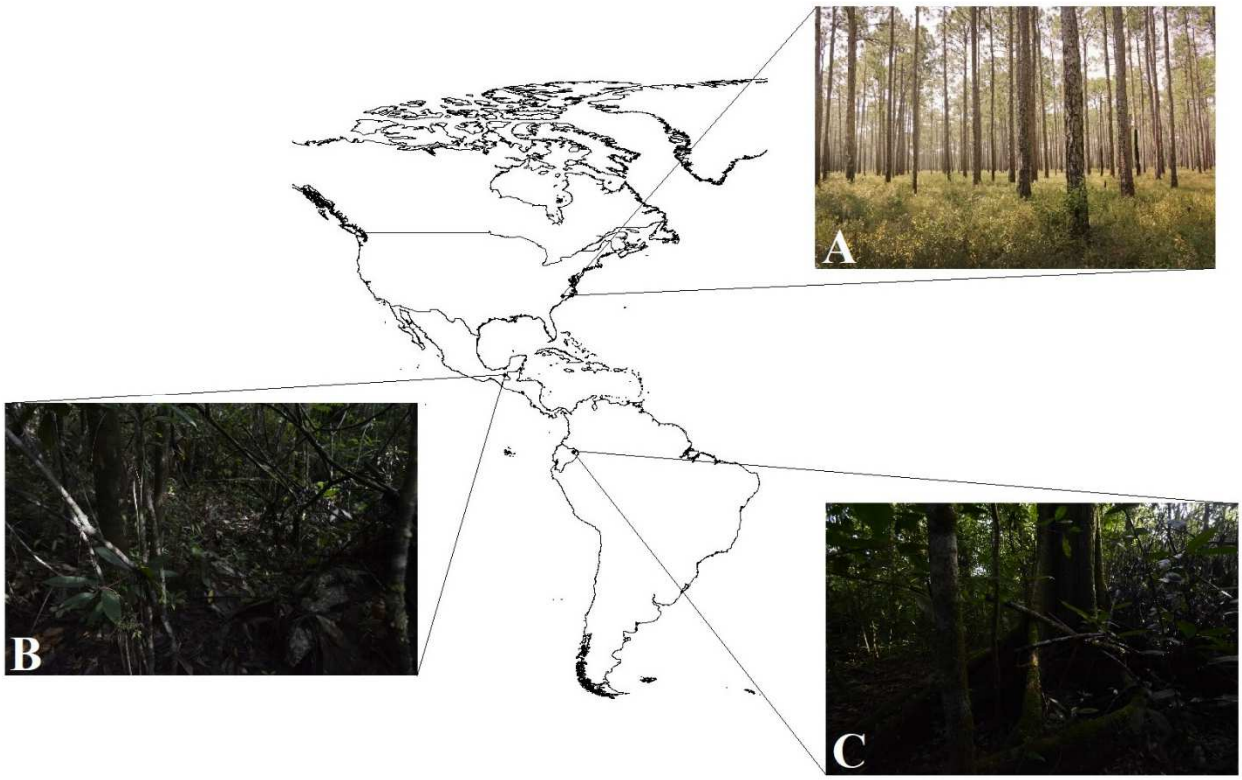

Figure 4.4. Detection and attack probabilities of avian versus mammalian predators. The probability that avian versus mammalian predators detected replicas after encounter (top) and attacked replicas after detection (bottom) across all experimental locations.

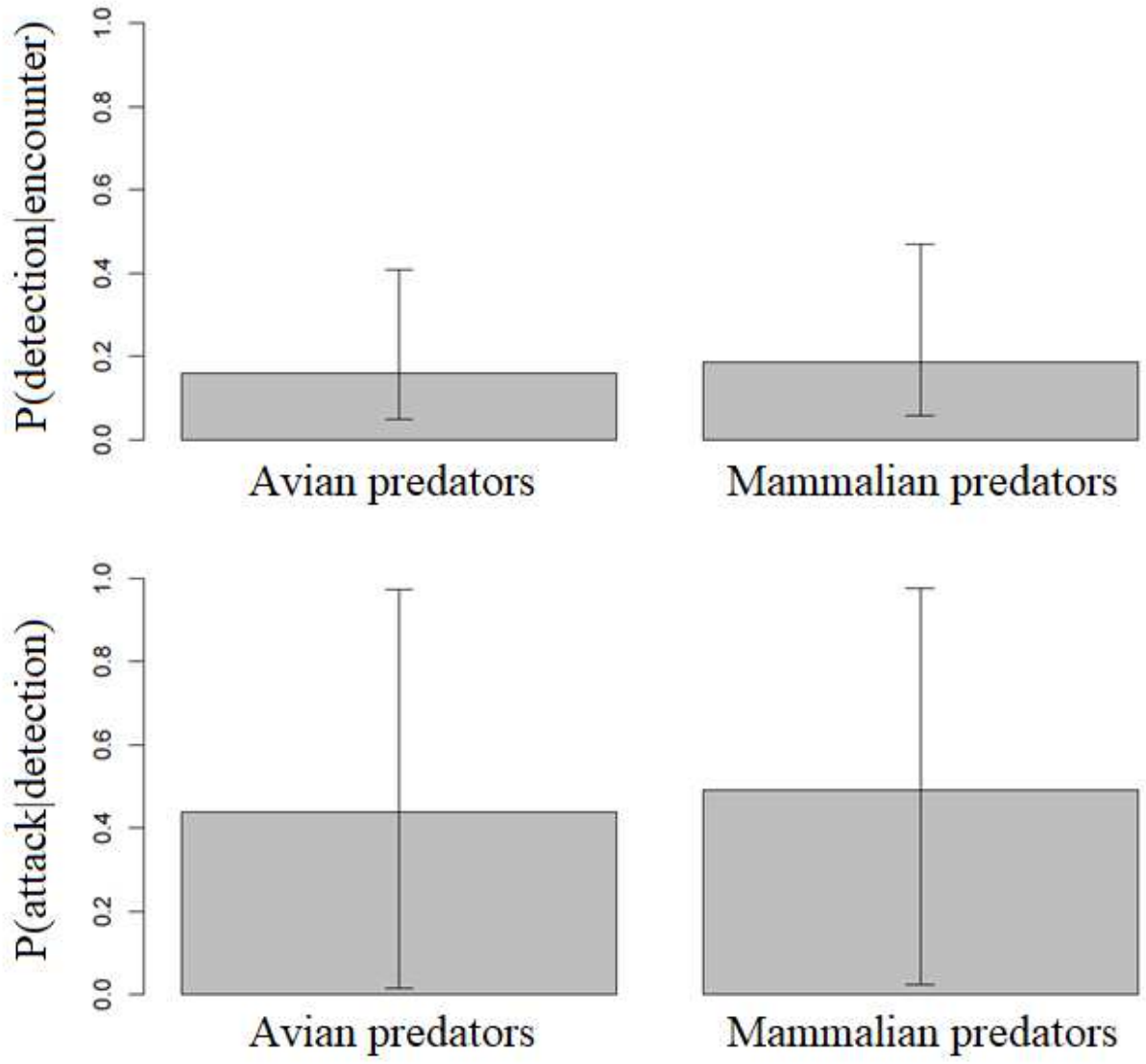

Figure 4.5. Observations vs. camera trapping effort. Frequency of encounters, detections, and attacks observed from camera trap videos monitoring artificial prey in Ecuador (402 camera days), Mexico (630 camera days), and North Carolina, USA (504 camera days), as a function of the number of camera days. Each point represents the frequency of either encounters, detections, or attacks from one of the field experimental locations. All regression lines were constrained to have an intercept through the origin.

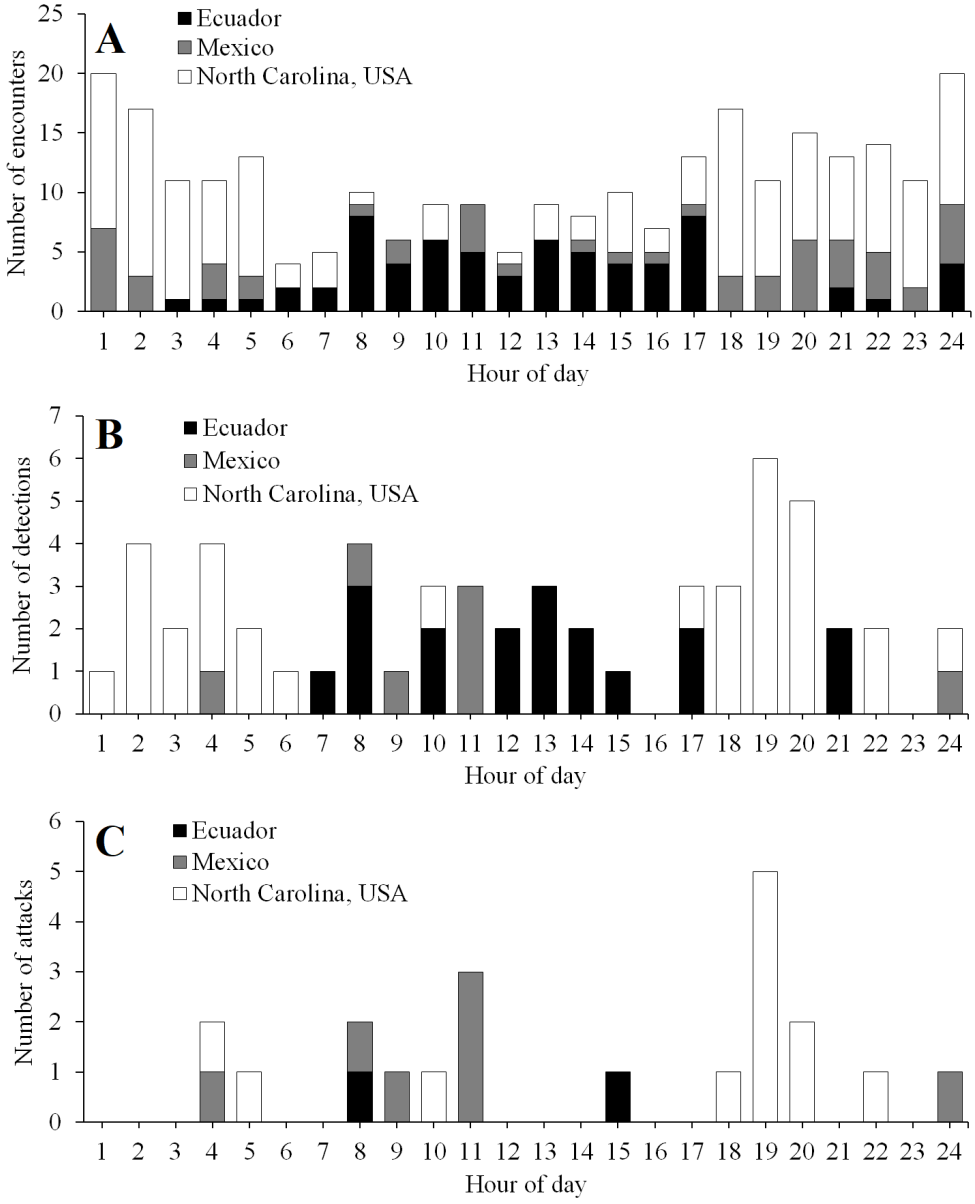

Figure 4.6. Temporal activity patterns. Diurnal patterns in the frequency of encounters (A), detections (B), and attacks (C) in field experiments conducted in Ecuador, Mexico, and North Carolina, USA. Daytime ran from 6 to 18, 6 to 19, and 8 to 17 hours in Ecuador, Mexico, and North Carolina, USA, respectively.

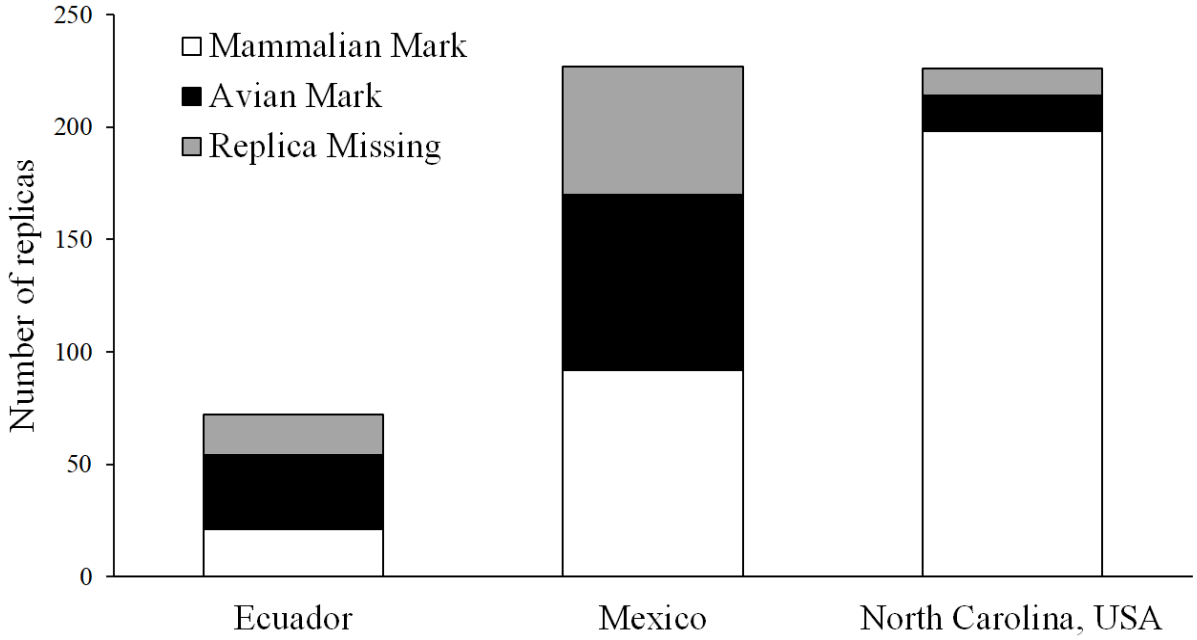

Figure 4.7. Results of field experiments. Numbers of replicas—both with and without camera traps—that bore marks indicative of attacks by avian and mammalian predators as well as numbers of replicas that were missing (i.e., no trace of replica found) in field experiments conducted in Ecuador, Mexico, and North Carolina, USA.

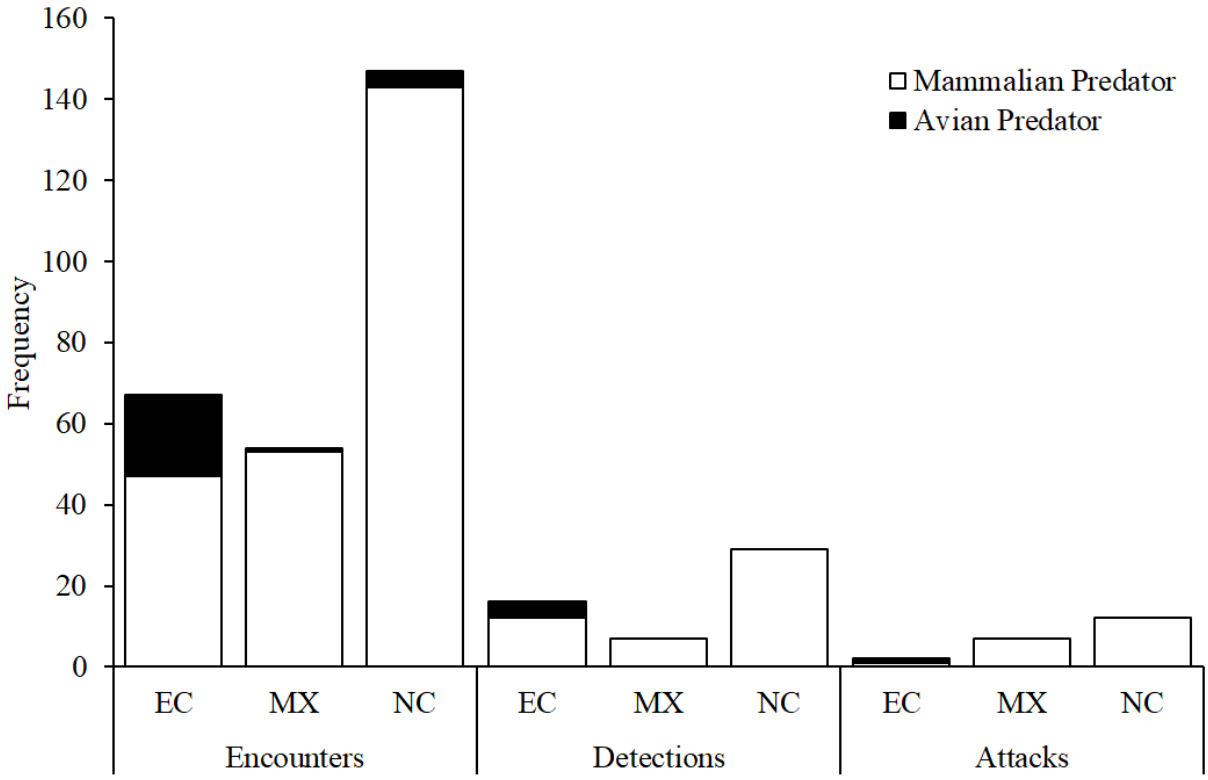

Figure 4.8. Camera trap observations. Numbers of encounters, detections, and attacks by avian and mammalian snake predators observed from camera trap videos at each experimental location: Ecuador (EC), Mexico (MX), and North Carolina, USA (NC).

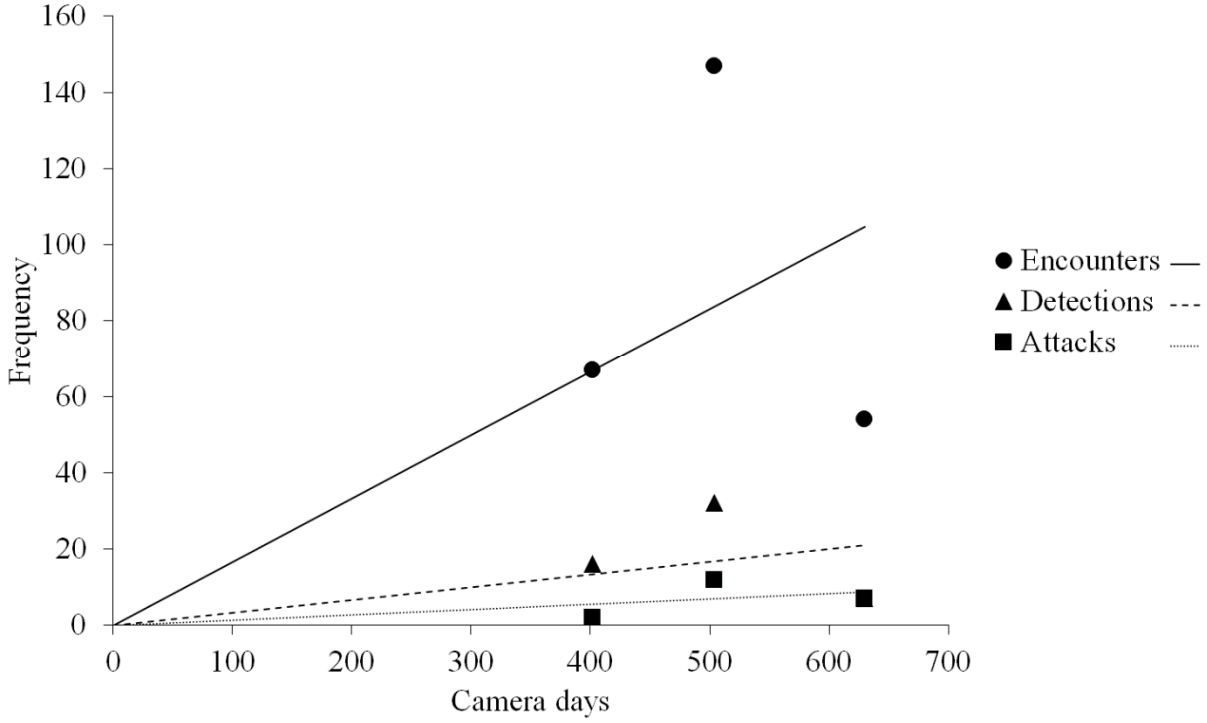

Figure 4.9. Costs of additional information. The costs of obtaining additional encounters (blue), detections (red), and attacks (green) for an artificial prey experiment that consists of 30 replicas, all monitored by cameras, that are exposed to natural predators for 12 days as a function of the predator activity level (% predation per day as a proxy) estimated from each of 424 artificial prey studies. Differences in the rates of encounters, detections, and attacks from other studies were assumed to be proportional to the rates of encounters, detections, and attacks estimated from this study. Black lines show the minimum predator activity level that would be necessary for the purchase of one additional $100 camera to capture an additional encounter, detection, or attack.